

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

|  |           |   |
|--|-----------|---|
| (51) International Patent Classification 7 :<br><b>C07H 21/04, C07K 1/00, C12P 12/06</b> | <b>A1</b> | (11) International Publication Number: <b>WO 00/17222</b>     |
|  |           | (43) International Publication Date: 30 March 2000 (30.03.00) |

(21) International Application Number: PCT/US99/22012

(22) International Filing Date: 22 September 1999 (22.09.99)

## (30) Priority Data:

|            |                              |    |
|------------|------------------------------|----|
| 60/101,546 | 23 September 1998 (23.09.98) | US |
| 60/102,895 | 2 October 1998 (02.10.98)    | US |

(71) Applicant (for all designated States except US): HUMAN GENOME SCIENCES, INC. [US/US]; 9410 Key West Avenue, Rockville, MD 20850 (US).

## (72) Inventors; and

(75) Inventors/Applicants (for US only): RUBEN, Steven, M. [US/US]; 18528 Heritage Hills Drive, Olney, MD 20832 (US). ROSEN, Craig, A. [US/US]; 22400 Rolling Hill Road, Laytonsville, MD 20882 (US). DUAN, Roxanne, D. [US/US]; 5515 Northfield Road, Bethesda, MD 20817 (US). SHI, Yanggu [CN/US]; Apartment 102, 437 West Side Drive, Gaithersburg, MD 20878 (US). LAFLEUR, David, W. [US/US]; 3142 Quesada Street, NW, Washington, DC 20015 (US). YOUNG, Paul, E. [US/US]; 122 Beckwith Street, Gaithersburg, MD 20878 (US). NI, Jian [US/US]; 5502 Manorfield Road, Rockville, MD 20853 (US). KOMATSOUKIS, George [US/US]; 9518 Garwood Street, Silver Spring, MD 20901 (US). ENDRESS, Gregory,

A. [US/US]; 9729 Clagett Farm Drive, Potomac, MD 20854 (US). SOPPET, Daniel, R. [US/US]; 15050 Stillfield Place, Centreville, VA 22020 (US).

(74) Agents: HOOVER, Kenley, K. et al.; Human Genome Sciences, Inc., 9410 Key West Avenue, Rockville, MD 20850 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published***With international search report.**Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

(54) Title: 31 HUMAN SECRETED PROTEINS

## (57) Abstract

The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human secreted proteins.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

|    |                          |    |  |    |  |    |                          |
|----|--------------------------|----|--|----|--|----|--------------------------|
| AL | Albania                  | ES | Spain                                    | LS | Lesotho                                      | SI | Slovenia                 |
| AM | Armenia                  | FI | Finland                                  | LT | Lithuania                                    | SK | Slovakia                 |
| AT | Austria                  | FR | France                                   | LU | Luxembourg                                   | SN | Senegal                  |
| AU | Australia                | GA | Gabon                                    | LV | Latvia                                       | SZ | Swaziland                |
| AZ | Azerbaijan               | GB | United Kingdom                           | MC | Monaco                                       | TD | Chad                     |
| BA | Bosnia and Herzegovina   | GE | Georgia                                  | MD | Republic of Moldova                          | TG | Togo                     |
| BB | Barbados                 | GH | Ghana                                    | MG | Madagascar                                   | TJ | Tajikistan               |
| BE | Belgium                  | GN | Guinea                                   | MK | The former Yugoslav<br>Republic of Macedonia | TM | Turkmenistan             |
| BF | Burkina Faso             | GR | Greece                                   | ML | Mali   | TR | Turkey                   |
| BG | Bulgaria                 | HU | Hungary                                  | MN | Mongolia                                     | TT | Trinidad and Tobago      |
| BJ | Benin                    | IE | Ireland                                  | MR | Mauritania                                   | UA | Ukraine                  |
| BR | Brazil                   | IL | Israel                                   | MW | Malawi                                       | UG | Uganda                   |
| BY | Belarus                  | IS | Iceland                                  | MX | Mexico                                       | US | United States of America |
| CA | Canada                   | IT | Italy                                    | NE | Niger  | UZ | Uzbekistan               |
| CF | Central African Republic | JP | Japan                                    | NL | Netherlands                                  | VN | Viet Nam                 |
| CG | Congo                    | KE | Kenya                                    | NO | Norway                                       | YU | Yugoslavia               |
| CH | Switzerland              | KG | Kyrgyzstan                               | NZ | New Zealand                                  | ZW | Zimbabwe                 |
| CI | Côte d'Ivoire            | KP | Democratic People's<br>Republic of Korea | PL | Poland                                       |    |                          |
| CM | Cameroon                 | KR | Republic of Korea                        | PT | Portugal                                     |    |                          |
| CN | China                    | KZ | Kazakstan                                | RO | Romania                                      |    |                          |
| CU | Cuba                     | LC | Saint Lucia                              | RU | Russian Federation                           |    |                          |
| CZ | Czech Republic           | LI | Liechtenstein                            | SD | Sudan  |    |                          |
| DE | Germany                  | LK | Sri Lanka                                | SE | Sweden                                       |    |                          |
| DK | Denmark                  | LR | Liberia                                  | SG | Singapore                                    |    |                          |
| EE | Estonia                  |    |  |    |  |    |                          |

## 31 Human Secreted Proteins

### *Field of the Invention*

This invention relates to newly identified polynucleotides and the polypeptides encoded by these polynucleotides, uses of such polynucleotides and polypeptides, and their production.

### *Background of the Invention*

Unlike bacterium, which exist as a single compartment surrounded by a membrane, human cells and other eucaryotes are subdivided by membranes into many functionally distinct compartments. Each membrane-bounded compartment, or organelle, contains different proteins essential for the function of the organelle. The cell uses "sorting signals," which are amino acid motifs located within the protein, to target proteins to particular cellular organelles.

One type of sorting signal, called a signal sequence, a signal peptide, or a leader sequence, directs a class of proteins to an organelle called the endoplasmic reticulum (ER). The ER separates the membrane-bounded proteins from all other types of proteins. Once localized to the ER, both groups of proteins can be further directed to another organelle called the Golgi apparatus. Here, the Golgi distributes the proteins to vesicles, including secretory vesicles, the cell membrane, lysosomes, and the other organelles.

Proteins targeted to the ER by a signal sequence can be released into the extracellular space as a secreted protein. For example, vesicles containing secreted proteins can fuse with the cell membrane and release their contents into the extracellular space - a process called exocytosis. Exocytosis can occur constitutively or after receipt of a triggering signal. In the latter case, the proteins are stored in secretory vesicles (or secretory granules) until exocytosis is triggered. Similarly, proteins residing on the cell membrane can also be secreted into the extracellular space by proteolytic cleavage of a "linker" holding the protein to the membrane.

Despite the great progress made in recent years, only a small number of genes encoding human secreted proteins have been identified. These secreted proteins include the commercially valuable human insulin, interferon, Factor VIII, human

growth hormone, tissue plasminogen activator, and erythropoietin. Thus, in light of the pervasive role of secreted proteins in human physiology, a need exists for identifying and characterizing novel human secreted proteins and the genes that encode them. This knowledge will allow one to detect, to treat, and to prevent  
5 medical disorders by using secreted proteins or the genes that encode them.

### *Summary of the Invention*

The present invention relates to novel polynucleotides and the encoded polypeptides. Moreover, the present invention relates to vectors, host cells,  
10 antibodies, and recombinant and synthetic methods for producing the polypeptides and polynucleotides. Also provided are diagnostic methods for detecting disorders and conditions related to the polypeptides and polynucleotides, and therapeutic methods for treating such disorders and conditions. The invention further relates to screening methods for identifying binding partners of the polypeptides.

15

### *Detailed Description*

#### Definitions

The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

20 In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of  
25 matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA preparations or other compositions where the art demonstrates no distinguishing  
30 features of the polynucleotide/sequences of the present invention.



In the present invention, a "secreted" protein refers to those proteins capable of being directed to the ER, secretory vesicles, or the extracellular space as a result of a signal sequence, as well as those proteins released into the extracellular space without necessarily containing a signal sequence. If the secreted protein is released  
5 into the extracellular space, the secreted protein can undergo extracellular processing to produce a "mature" protein. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage.

In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000  
10 continuous nucleotides but are less than or equal to 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, 7.5 kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment, polynucleotides of the invention comprise a portion of the coding sequences, as disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding  
15 sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

As used herein, a "polynucleotide" refers to a molecule having a nucleic acid  
20 sequence contained in SEQ ID NO:X or the cDNA contained within the clone deposited with the ATCC. For example, the polynucleotide can contain the nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without the signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants  
25 of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a molecule having the translated amino acid sequence generated from the polynucleotide as broadly defined.

In the present invention, the full length sequence identified as SEQ ID NO:X was often generated by overlapping sequences contained in multiple clones (contig  
30 analysis). A representative clone containing all or most of the sequence for SEQ ID NO:X was deposited with the American Type Culture Collection ("ATCC"). As

shown in Table 1, each clone is identified by a cDNA Clone ID (Identifier) and the ATCC Deposit Number. The ATCC is located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:X, the complement thereof, or the cDNA within the clone deposited with the ATCC. "Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C.

Also contemplated are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M  $\text{NaH}_2\text{PO}_4$ ; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 µg/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking

reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA+ sequences (such as any 3' terminal polyA+ tract of a cDNA shown in the sequence listing), or to a  
5 complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using dT as a primer).

The polynucleotide of the present invention can be composed of any  
10 polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA  
15 that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for  
20 example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

The polypeptide of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide  
25 isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications  
30 can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same

type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann NY Acad Sci 663:48-62 (1992).)

"SEQ ID NO:X" refers to a polynucleotide sequence while "SEQ ID NO:Y" refers to a polypeptide sequence, both sequences identified by an integer specified in Table 1.

"A polypeptide having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less

activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention.)

### **Polynucleotides and Polypeptides of the Invention**

#### **5 FEATURES OF PROTEIN ENCODED BY GENE NO: 1**

The gene encoding the disclosed cDNA is believed to reside on chromosome 12. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 12.

10 This gene is expressed primarily in brain, colon, adipose tissue, fetal tissue, and to a lesser extent, in ovary and testes.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurodegenerative disorders; learning disabilities; glioblastomas; 15 impaired muscle function; reproductive disorders; fertility problems. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the nervous and reproductive systems, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g., neural, endocrine, reproductive, 20 endocrine, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, seminal fluid, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder. 25

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 48 as residues: Ser-25 to Lys-30. Polynucleotides encoding said polypeptides are also provided.

30 The tissue distribution in brain indicates polynucleotides and polypeptides corresponding to this gene are useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions.

Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function.

Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Elevated expression of this gene product in regions of the brain also indicates that it plays a role in normal neural function. Additionally, expression of this gene product in reproductive organs such as ovary and testes indicates that it may play additional roles in reproductive function, or in the development or maturation of sex cells.

The tissue distribution in adipose tissue indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment of obesity and other metabolic and endocrine conditions or disorders. Furthermore, the protein product of this gene may show utility in ameliorating conditions which occur secondary to aberrant fatty-acid metabolism (e.g. aberrant myelin sheath development), either directly or indirectly. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are

related to SEQ ID NO:11 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1993 of SEQ ID NO:11, b is an integer of 15 to 2007, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:11, and where b is greater than or equal to a + 14.

## 10 FEATURES OF PROTEIN ENCODED BY GENE NO: 2

The gene encoding the disclosed cDNA is thought to reside on chromosome 19. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 19.

Preferred polypeptides comprise the following amino acid sequence:

15 PPALGPVSPGASGSPGPVAAAPSSLVAAAASVAAAAGGDLGWMAETAIIITD  
ASFLSGLSASLLERRPASPLGPAGGLPHAPQDSVPPSDSAASDTTPLGAAVGG  
PSPASMAPTEAPSEVGS (SEQ ID NO: 85);  
KSVKLVRLQVPVRNSRVDPRVRKGFLRNVVSGEHYRFVSMWMARTSYLAA  
FAIMVIFTLSVSMLLRYSHHQIFVFIAPLLTVILALVGMEAIMSEFFNDTTTAFY  
20 IILIVWLADQYDAICCHTSTSKRHWLRFYLYHFAYYHYRFNGQYSSLALV  
TSWLFIQHSMIYFFHHYELPAILQQVRIQEMLLQAPPLGPGTPTALPDDMNNN  
SGAPATAPDSAGQPPALGPVSPGASGSPGPVAAAPSSLVAAAASVAAAAGGD  
LGWMAETAIIITDASFLSGLSASLLERRPASPLGPAGGLPHAPQDSVPPSDSA  
ASDTTPLGAAVGGPSPASMAPTEAPSEVGS (SEQ ID NO: 86);  
25 MGPHSILRTVHCRPTKTPPEPSAEPHPLSLLTSSNTSLAGTSLGRDLTPGGGKP  
PSGQTPRNPESPRHRLGSPRGRRWLASPTPTGSGRSGPASRGQRRLSCAAQDP  
TSEGASVGAMEAGLGPPTAAPRGVVSEAAESLGGTLSWGAWGRPPAGPSGL  
AGRRSRREALRPDRKEASVMMAAVSAIQPRSPAAAATEAAAATRELGAAA  
TGPGPLAPGETGPRAGGWPAESGAVAGAPELLFMSSGSAVGVPGPSGGA  
30 (SEQ ID NO: 87); and  
MSAPPHSSPSDWFGRRPTSPSPSGTGPRPWLLPLMLAPAPHVPMPEAQALLSRG

PQAWRTRGEGGAMEKALQGAPGRAGLRPAGTRARGPTPSRPLLHTSALLRD  
LHHGTPLHPQDGS LQTYQDPSRTFRGTPPPLLADQLKHLTSGYKPRARPHTR  
GRKAAFR ANPTKP (SEQ ID NO: 88). Also preferred are the polynucleotides  
encoding these polypeptides.

5           This gene is expressed primarily in hematopoietic cells and tissues, ovary  
tumor, parathyroid tumor, brain, immune tissues (e.g., eosinophils, bone marrow,  
dendritic cells) and to a lesser extent in breast cancer, epithelial cells, and Hodgkin's  
lymphoma.

          Therefore, polynucleotides and polypeptides of the invention are useful as  
10   reagents for differential identification of the tissue(s) or cell type(s) present in a  
biological sample and for diagnosis of diseases and conditions which include, but are  
not limited to, hematopoietic disorders; impaired immunity; immune dysfunction;  
breast and ovarian cancer; disorders of the central nervous system; wound healing;  
Hodgkin's lymphoma. Similarly, polypeptides and antibodies directed to these  
15   polypeptides are useful in providing immunological probes for differential  
identification of the tissue(s) or cell type(s). For a number of disorders of the above  
tissues or cells, particularly of the immune system and central nervous system,  
expression of this gene at significantly higher or lower levels is routinely detected in  
certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily  
20   fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another  
tissue or cell sample taken from an individual having such a disorder, relative to the  
standard gene expression level, i.e., the expression level in healthy tissue or bodily  
fluid from an individual not having the disorder.

          Preferred polypeptides of the present invention comprise immunogenic  
25   epitopes shown in SEQ ID NO: 49 as residues: Ser-71 to Trp-77, Tyr-91 to Tyr-99,  
Asp-153 to Gly-160, Ala-165 to Gly-170, Ala-252 to Ala-264. Polynucleotides  
encoding said polypeptides are also provided.

          The tissue distribution in hematopoietic tissues indicates that polynucleotides  
and polypeptides corresponding to this gene are useful for the diagnosis and/or  
30   treatment of a variety of hematopoietic disorders. Elevated expression of this gene  
product in a variety of immune cell types and tissues including dendritic cells, T cells,



GKDCEGPRSVQDGGQPTVLQDGFKA AEGLGRGEDLS DSSLGIPRGPRGGLPP  
QASDHVEDAISCYVVGLVDVERLLGLVFVLVGVFREL VKGDGDLLPGQRPPP  
SCLLGPCVLQDVLPCDDVLSTELLGKGCIHGPGGEGGDGWHQHGERARCGK  
DFPAALVHHGHGDPQLQEHPACLRGLLHLVEQVSVAGTPIPHLASLHTQLVF  
5 PSAVTWQVADRSQEKGEQKAKQPAEALLMLTRPGRQVQKAWPGFWYTHK  
VELLGQPPRLSVHGSLGRHTIPSPW (SEQ ID NO: 90). Also preferred are the  
polynucleotides encoding these polypeptides.

The gene encoding the disclosed cDNA is believed to reside on chromosome  
3. Accordingly, polynucleotides related to this invention are useful as a marker in  
10 linkage analysis for chromosome 3.

This gene is expressed primarily in primary dendritic cells, T-cells, spleen,  
lymph node, fetal liver, and blood leukocyte, thymus, peripheral blood, leukocyte,  
bone marrow, fetal liver, prostate, testis, uterus, small intestine, colon, peripheral  
blood, leukocyte, adult liver, fetal liver, fetal spleen, heart, placenta, lung, adrenal  
15 glands, peripheral leukocyte, bone marrow, and appendix.

Therefore, polynucleotides and polypeptides of the invention are useful as  
reagents for differential identification of the tissue(s) or cell type(s) present in a  
biological sample and for diagnosis of diseases and conditions which include, but are  
not limited to, immune dysfunction; impaired immunity; autoimmunity;  
20 hematopoietic disorders; impaired antigen presentation. Similarly, polypeptides and  
antibodies directed to these polypeptides are useful in providing immunological  
probes for differential identification of the tissue(s) or cell type(s). For a number of  
disorders of the above tissues or cells, particularly of the immune system, expression  
of this gene at significantly higher or lower levels is routinely detected in certain  
25 tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids  
(e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or  
cell sample taken from an individual having such a disorder, relative to the standard  
gene expression level, i.e., the expression level in healthy tissue or bodily fluid from  
an individual not having the disorder.

30 Preferred polypeptides of the present invention comprise immunogenic  
epitopes shown in SEQ ID NO: 50 as residues: Glu-62 to Gln-69, Thr-96 to Lys-103.

Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in hematopoietic and immune cells and homology to EGF-like motifs indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of a variety of hematopoietic or immune disorders. Expression of this gene product selectively on dendritic cells indicates that it is involved in immune recognition. Potentially, it participates in antigen presentation, or in the costimulation of T- and B-cells during antigen recognition. Alternately, it is involved in the trafficking or motility of antigen presenting cells, and in their homing to sites of infection or inflammation.

Additionally, the tissue distribution in immune cells and immune tissues (e.g., bone marrow and leukocytes) indicates polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "infectious disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses).

Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous Disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's Disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful

in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Moreover, the expression within fetal tissue (e.g., fetal liver and spleen) and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation.

Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases.

The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly

available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:13 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
5 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2794 of SEQ ID NO:13, b is an integer of 15 to 2808, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:13, and where b is greater than or equal to a + 14.

10

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 4

This gene is expressed primarily in primary dendritic cells, fetal brain, kidney, pancreatic islet cells, ovarian cancer, T-cells, and to a lesser extent, in prostate.

Therefore, polynucleotides and polypeptides of the invention are useful as  
15 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, prostate cancer; immune dysfunction; autoimmunity; susceptibility to infection; neurodegenerative disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for  
20 differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune or nervous systems, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g., immune, neural, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another  
25 tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 51 as residues: Ser-42 to Asp-51, Gln-59 to Ser-64.

30 Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in immune cells indicates polynucleotides and

polypeptides corresponding to this gene are useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "infectious disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses).

Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous Disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's Disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Additionally, expression of this gene product within the fetal brain indicates a role in the differentiation of early neural networks within the developing brain, or in neuronal survival. Such roles would suggest useful clinical uses for this gene product in the treatment of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease,

Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function.

Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Expression of this gene product in prostate may also be a diagnostic marker for the development of prostate cancer, or may indicate a normal role for this gene product in normal prostate function. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:14 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1375 of SEQ ID NO:14, b is an integer of 15 to 1389, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:14, and where b is greater than or equal to a + 14.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 5**

A preferred polypeptide variant of the invention comprises the following amino acid sequence:

MWSLVSVSVLVLTCAMDVAEGLGWGEVSTGGIELPRHMLVVLVERESQRX

5 RTCSVKTFSSR (SEQ ID NO: 91). Polynucleotides encoding these polypeptides are also provided.

This gene is expressed primarily in brain frontal cortex.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neurodegenerative disorders; learning disabilities; impaired neurotransmission; muscular weakness; compulsive disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the brain and nervous system, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g. brain, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 52 as residues: Pro-59 to Ser-68. Polynucleotides encoding said polypeptides are also provided.

25 The tissue distribution in brain frontal cortex indicates polynucleotides and polypeptides corresponding to this gene are useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease,

Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function.

Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:15 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1859 of SEQ ID NO:15, b is an integer of 15 to 1873, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:15, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 6

The translation product of this gene shares sequence homology with alpha 2,6-sialyltransferase (see, e.g., Genbank accession numbers CAB 44338.1 (Y17466.1) and AAC42086.1 (L29554.1); all references available through this accession are hereby incorporated by reference herein.) which is thought to be important in the



glycosylation of various proteins.

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 9-25 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 26-319  
5 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type Ib membrane proteins.

This gene is expressed primarily in T cells, brain, eosinophils, ovarian tumors, osteoblasts, fetal tissue (e.g., lung) and to a lesser extent in bone cells, epithelial cells,  
10 and many other tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, impaired immune function; neurodegenerative disorders; learning  
15 disabilities; hematopoietic disorders; osteoporosis; osteopetrosis; non-healing wounds and ulcerations. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and nervous systems, expression of this gene at  
20 significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not  
25 having the disorder.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 53 as residues: Met-1 to Arg-8, Leu-39 to Asn-48, Lys-65 to Cys-71, Met-101 to Tyr-109, Val-113 to Thr-118, Arg-132 to Phe-137, Pro-151 to Gln-159, Gly-184 to Phe-189, Glu-196 to Ser-204, Asn-234 to Arg-244, Asn-  
30 264 to Asn-271, Gly-308 to Arg-319. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution and homology to glycosyl-transferases indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of a variety of disorders. Glycosylation of proteins can affect many different biological processes. For example, glycosylation of certain cell surface proteins is critical for immune surveillance and inflammation, and the recognition of such carbohydrate structures by proteins such as the selectins mediates neutrophil extravasation. Similarly, defects in correct glycosylation can lead to various diseases and syndromes, such as Wiskott-Aldrich. Expression of this glycosyltransferase within hematopoietic cells, brain, bone, and epithelial cells, indicates that it may play key roles in the survival, proliferation, differentiation, or activation of blood cells; in neuronal survival or synapse formation; in learning; in bone metabolism and osteoporosis/osteopetrosis; and in natural wound healing.

Additionally, the tissue distribution in brain indicates polynucleotides and polypeptides corresponding to this gene are useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function.

Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. The tissue distribution in eosinophils and T-cells also indicates polynucleotides and polypeptides corresponding to this gene are useful for the

diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "infectious disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses).

Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous Disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's Disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Moreover, the expression within fetal tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation.

Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent

of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:16 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2395 of SEQ ID NO:16, b is an integer of 15 to 2409, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:16, and where b is greater than or equal to a + 14.

### **30 FEATURES OF PROTEIN ENCODED BY GENE NO: 7**

The polypeptide of this gene has been determined to have a transmembrane

domain at about amino acid position 2-18 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 19-96 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type Ib membrane proteins.

This gene is expressed primarily in hematopoietic cells and tissues, fetal tissue (e.g., liver, spleen), brain, bone marrow, and to a lesser extent in endothelial cells, such as aortic endothelial cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune system dysfunction; autoimmunity; impaired immunity; hematopoietic disorders; neutropenia; lymphomas; aberrant angiogenesis; hemangiomas. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and circulatory systems, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g., immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, blood, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 54 as residues: Gly-79 to Trp-91. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in bone marrow indicates polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "infectious disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene

product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses).

Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous Disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's Disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Alternately, expression of this gene product in endothelial cells indicates a role in the development of the vasculature or in angiogenesis. Alternately, expression of this gene product in endothelial cells may suggest a simple source for this secreted protein into the circulation, where it may have effects on a variety of different tissues.

The tissue distribution in brain indicates polynucleotides and polypeptides corresponding to this gene are useful for the detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia,

trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep  
5 patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function.

Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity,  
10 raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly  
15 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:17 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
20 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1576 of SEQ ID NO:17, b is an integer of 15 to 1590, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:17, and where b is greater than or equal to a + 14.

## 25 **FEATURES OF PROTEIN ENCODED BY GENE NO: 8**

The polypeptide of this gene has been determined to have transmembrane domains at about amino acid position 177-193, 218-234, 318-334, 261-277, 52-68, and 295-311 of the amino acid sequence referenced in Table 1 for this gene. Based upon these characteristics, it is believed that the protein product of this gene shares  
30 structural features to type IIIa membrane proteins.

This gene is expressed primarily in JURKAT T-cells, multiple sclerosis tissue

and rejected kidney.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, leukemia, transplantation, renal, or immune disorders and/or multiple sclerosis. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g., immune, renal, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 55 as residues: Cys-36 to Phe-44, Ala-72 to Ser-78. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in T-cells indicates that polynucleotides and polypeptides corresponding to this gene are useful for leukemia and transplantation. Likewise, expression of this gene product in T-cells indicates a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. Representative uses are described in the "Immune Activity" and "infectious disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses).

Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such



as AIDS, leukemia, rheumatoid arthritis, granulomatous Disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's Disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Alternatively, the expression of this gene product in kidney indicates that this gene or gene product could be used in the treatment and/or detection of kidney diseases including renal failure, nephritis, renal tubular acidosis, proteinuria, pyuria, edema, pyelonephritis, hydronephritis, nephrotic syndrome, crush syndrome, glomerulonephritis, hematuria, renal colic and kidney stones, in addition to Wilms Tumor Disease, and congenital kidney abnormalities such as horseshoe kidney, polycystic kidney, and Falconi's syndrome. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:18 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1553 of SEQ ID NO:18, b is an integer of 15 to 1567, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:18, and where b is greater than or equal to a + 14.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 9**

The translation product of this gene shares sequence homology with a C. elegans transposase and a transposase from Magnaporthe grisea, which is thought to be important in the movement of transposable elements from one genomic location to another, which is related to cancer induction.

The gene encoding the disclosed cDNA is thought to reside on chromosome 1. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 1.

This gene is expressed primarily in fetal tissues including infant brain, six week old embryo, fetal cochlea, fetal spleen, placenta, general fetal tissue uninduced umbilical vein endothelial cells, 12 week old early stage human (I and II), and nine week old early stage human, and to a lesser extent in a variety of normal and transformed tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer and other proliferative disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 56 as residues: Met-1 to Asp-30, Pro-44 to Gly-49, Arg-76 to Gly-85, Glu-95 to Cys-100, Asn-112 to Leu-123, Arg-134 to Arg-140, Arg-193 to Asp-200, Pro-273 to Leu-278, Asp-289 to Pro-294, Arg-316 to Trp-322, Trp-

336 to Gly-341, Asn-365 to Asn-371, Thr-418 to Pro-424, Ser-456 to Asp-462, Pro-473 to Asp-483. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution and homology to a *C. elegans* transposase indicates that polynucleotides and polypeptides corresponding to this gene are useful for the  
5 detection and treatment of cancer and other proliferative disorders, since it is believed that some transposon insertions can affect growth control. Expression of this gene product in progenitors cells of immune function indicates a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product is involved  
10 in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses).

Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also used as an agent for  
15 immunological disorders including arthritis, asthma, immune deficiency diseases such as AIDS, leukemia, rheumatoid arthritis, inflammatory bowel disease, sepsis, acne, and psoriasis. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Protein, as well as,  
20 antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:19 and may have been publicly available prior to conception of  
25 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3416 of SEQ ID NO:19, b is an  
30 integer of 15 to 3430, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:19, and where b is greater than or equal to a + 14.

# FEATURES OF PROTEIN ENCODED BY GENE NO: 10

The gene encoding the disclosed cDNA is believed to reside on chromosome 3. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 3.

The polypeptide of this gene has been determined to have transmembrane domains at about amino acid positions 119-135, 66-82, 48-64, and 101-117 of the amino acid sequence referenced in Table 1 for this gene. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type IIIb membrane proteins.

A preferred polypeptide fragment of the invention comprises the following amino acid sequence:

MILGGIVVVLVFTGFVWAAHNKDVLRMKKRYPTTFVMVVMLASYFLISMF  
GGVMVFVFGITFPLLLMFIHASLRLRLNKNKLENKMEGIGLKRTPMGIVLDAL  
EQQEEGINRLTDYISKVKE (SEQ ID NO: 92). Polynucleotides encoding these polypeptides are also provided.

This gene is expressed primarily in immune, tumor and fetal tissues. Immune expression includes primary dendritic cells, T cell helper II, Apoptotic T-cell, T-Cell PHA 24 hrs, CD34+cells, II, FRACTION 2, CD34+cells, II, FRACTION 2, CD34 depleted Buffy Coat (Cord Blood), H Macrophage (GM-CSF treated), re-excision, T Cell helper I, Macrophage-oxLDL; re-excision, Human Neutrophil, Activated, CD34 depleted Buffy Coat (Cord Blood), re-excision, Anergic T-cell, Monocyte activated, Neutrophils IL-1 and LPS induced. Tumors expressing this gene include endometrial, osteoclastoma and osteoclastoma (re-excision), T-cell lymphoma (re-excision), chondrosarcoma, stage B2 prostate cancer, NTERA2 teratocarcinoma cell line+retinoic acid (14 days), ovarian, islet cell, T-cell lymphoma, Human (Caco-2) cell line, adenocarcinoma, Pancreas Tumor PCA4 Tu, colon cancer (re-excision). Fetal expression occurs in Nine Week Old Early Stage Human, placenta, liver spleen, Human 8 Week Whole Embryo ,lung, 12 Week Old Early Stage Human, heart, brain (subtracted), liver (subtracted) and to a lesser extent in a variety of normal and diseased adult tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, cancer and other proliferative disorders, disorders of the cytoskeleton and immune disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 57 as residues: Pro-16 to Phe-21, Pro-24 to Arg-35, Arg-92 to Pro-98, Asn-143 to Lys-151, Leu-169 to Ile-176. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution (the sequence is present in actively growing tissues such as cancers and fetal tissues, as well as immune tissues) indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of cancer and other proliferative disorders. Moreover, the expression within fetal tissue and other cellular sources marked by proliferating cells indicates this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis, treatment, and/or prevention of developmental diseases and disorders, including cancer, and other proliferative conditions. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Briefly, developmental tissues rely on decisions involving cell differentiation and/or apoptosis in pattern formation.

Dysregulation of apoptosis can result in inappropriate suppression of cell death, as occurs in the development of some cancers, or in failure to control the extent

of cell death, as is believed to occur in acquired immunodeficiency and certain neurodegenerative disorders, such as spinal muscular atrophy (SMA). Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Therefore, the polynucleotides and polypeptides of the present invention are useful in treating, detecting, and/or preventing said disorders and conditions, in addition to other types of degenerative conditions. Thus this protein may modulate apoptosis or tissue differentiation and is useful in the detection, treatment, and/or prevention of degenerative or proliferative conditions and diseases. The protein is useful in modulating the immune response to aberrant polypeptides, as may exist in proliferating and cancerous cells and tissues. The protein can also be used to gain new insight into the regulation of cellular growth and proliferation. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:20 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1515 of SEQ ID NO:20, b is an integer of 15 to 1529, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:20, and where b is greater than or equal to a + 14.

### 30 FEATURES OF PROTEIN ENCODED BY GENE NO: 11

The translation product of this gene shares sequence homology with a human

macrophage-specific FcRI receptor, which is thought to be important in the binding of this receptor to ligands.

This gene is expressed primarily in Hodgkins lymphoma II, B-cell lymphomas, and to a lesser extent in human testes.

5 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune disorders and cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for  
10 differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample  
15 taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to human macrophage-specific FcRI receptor indicates that polynucleotides and polypeptides corresponding to this gene  
20 are useful for increasing the potency of antibodies in therapy, if it were used as a fusion protein of the translation product of this gene and a receptor ligand. The tissue distribution in Hodgkins lymphomas and B-cell lymphomas indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and intervention of these tumors, in addition to other tumors where expression has  
25 been indicated. The tissue distribution in B-cell lymphomas also indicate polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "infectious disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the  
30 expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including

blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses).

Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous Disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's Disease, and scleroderma.

Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:21 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2411 of SEQ ID NO:21, b is an



integer of 15 to 2425, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:21, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 12

5 The translation product of this gene shares sequence homology with a rat uterus-specific transmembrane protein of unknown function (See, e.g., Genbank Accession No. gi|2460316|gb|AAB71895.1| (AF022147); all references available through this accession are hereby incorporated by reference herein), as well as to the tolloid-like family of proteins that contain repeating functional units of protein domains. These proteins are frequently involved in developmental decisions involving cell proliferation or differentiation.

Preferred polypeptides of the invention comprise the following amino acid sequences:

QLDPDGSCSENIKVFDGTSSNGPLLQGQVCSKNDYVPVFESSSSTLTFQIVTDS  
15 ARIQRTVFVFYFFSPNISIPNCGGYLDTLEGSFTSPNYPKPHPELAYCVWHIQ  
VEKDYKIKLNFKEIFLEIDKQCKFDLAIYDGPSTNSGLIGQVCGRVTPTFESS  
NSLTVVLSTDYANSYRGFSASYTSIYAENINTTSLTCSSDRMRVIISKSYLEAF  
NSNGNNLQLKDPTWQTKIIKWWGNFLVLLMDVVHSER (SEQ ID NO: 94);  
EAEGNASCTVSLGGANMAETHKAMILQLNPSENCTWTIERPENKSIRIIFS

20 (SEQ ID NO: 95); and/or

MPLTLLILSCLADWTMAEAEGNASCTVSLGGANMAETHKAMILQLNPSENC  
TWTIERPENKSIRIIFS YVQLDPDGSCSENIKVFDGTSSNGPLLQGQVCSKNDY  
VPVFESSSSTLTFQIVTDSARIQRTVFVFYFFSPNISIPNCGGYLDTLEGSFTSP  
NYPKPHPELAYCVWHIQVEKDYKIKLNFKEIFLEIDKQCKFDLAIYDGPSTNS  
25 GLIGQVCGRVTPTFESSNSLTVVLSTDYANSYRGFSASYTSIYAENINTTSLT  
CSSDRMRVIISKSYLEAFNSNGNNLQLKDPTWQTKIIKWWGNFLVLLMDVVH  
SER (SEQ ID NO: 96). Polynucleotides encoding such polypeptides are also

provided. Also provided are polypeptides comprising tolloid-like repetitive element with the following amino acid sequence:

30 CGGYLDTLEGSFTSPNYPKPHPELAYCVW (SEQ ID NO:141).

Further preferred are polypeptides comprising the tolloid-like repetitive

element listed above, and at least 5, 10, 15, 20, 25, 30, 50, or 75 additional contiguous amino acid residues of the amino acid sequence referenced in Table 1 for this gene.

The additional contiguous amino acid residues is N-terminal or C-terminal to the toll-like repetitive element. Alternatively, the additional contiguous amino acid residues is both N-terminal and C-terminal to the MIP family signature, wherein the total N- and C-terminal contiguous amino acid residues equal the specified number.

A preferred polypeptide fragment of the invention comprises the following amino acid sequence:

MPLTLLILSCLADWTMAEAEGNASCTVSLGGANMAETHKAMILQLNPSENC

TWTIERPENKSIRIIFSYPVA (SEQ ID NO: 93). Polynucleotides encoding these polypeptides are also provided.

This gene is expressed primarily in pancreas and pancreatic cancer.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, pancreatic cancer and tumors; diabetes. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the pancreas or endocrine system, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g., pancreas, cancerous and wounded tissues) or bodily fluids (e.g., lymph, bile, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 59 as residues: Asn-46 to Cys-51, Glu-56 to Ser-62, Asp-73 to Glu-79, Phe-158 to Pro-168, Glu-180 to Ile-185, Asp-209 to Asn-214, Phe-229 to Asn-234, Asp-243 to Arg-249, Asn-288 to Asn-293, Lys-297 to Gln-302.

Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in pancreas and pancreatic tumor tissue and homology

to tolloid family members indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and/or treatment of defects involving abnormal cellular proliferation or differentiation. Moreover, the tissue distribution in pancreas indicates that polynucleotides and polypeptides corresponding to this gene are useful for the treatment, prevention and/or diagnosis of disorders of the pancreas, including inflammatory disorders, such as chronic or acute pancreatitis; diabetes mellitus; pancreatic cancer. Specific expression of this gene product in the pancreas indicates that it is involved in the development or maintenance of specific pancreatic structures, such as the pancreatic islet cells. Alternately, as the pancreas is an endocrine organ, it may simply be produced in the pancreas, and have effects elsewhere in the body, for example, on epithelial cells, endothelial cells, fat cells, etc. Potentially, this gene is involved in the regulation of insulin production within the pancreas. Thus, this gene product may play various roles in the body, including effects on metabolism, obesity, homeostasis, neural function, hematopoiesis, etc.

The specific expression in pancreas and pancreatic cancer and predicted cell surface localization indicates that this gene is a good target for antagonists, particularly small molecules or antibodies, which may inhibit the biological function of this protein. Accordingly, preferred are antibodies and or small molecules which specifically bind an extracellular portion of the translation product of this gene. Also provided is a kit for detecting pancreatic cancer. Such a kit comprises in one embodiment an antibody specific for the translation product of this gene bound to a solid support. Also provided is a method of detecting pancreatic cancer in an individual which comprises a step of contacting an antibody specific for the translation product of this gene to a bodily fluid from the individual, preferably serum, and ascertaining whether antibody binds to an antigen found in the bodily fluid. Preferably the antibody is bound to a solid support and the bodily fluid is serum. The above embodiments, as well as other treatments and diagnostic tests (kits and methods), are more particularly described elsewhere herein.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:22 and may have been publicly available prior to conception of

the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general  
 5 formula of a-b, where a is any integer between 1 to 1957 of SEQ ID NO:22, b is an integer of 15 to 1971, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:22, and where b is greater than or equal to a + 14.

### FEATURES OF PROTEIN ENCODED BY GENE NO: 13

10 The translation product of this gene shares sequence homology with CTX, which is thought to be important in immunoregulation of thymocytes in *Xenopus*. (See Genbank Accession Nos. gi|1335866). Additionally, translation product of this gene shares sequence homology with the A33 gene, which is expressed in colon cells, and is thought to be important in immunoregulation of thymocytes or colon tissues (See  
 15 Genbank Accession No. gi|1814277; all referenced available through this accession are hereby incorporated by reference herein).

The gene encoding the disclosed cDNA is believed to reside on chromosome  
 11. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 11.

20 A preferred polypeptide fragment of the invention comprises the following amino acid sequence:  
 MAELPGPFLCGALLGFLCLSGLA VEKVPTEPLSTPLGKTAELTCTYSTSVGD  
 SFALEWSFVQPGKPISEHPILYFTNGHLYPTGSKSKRVSLQNPPTVGVATLK  
 LTDVHPSDTGTYLCQVNNPPDFYTNGLG LINLTVLVPPSNPLCSQSGQTSVGG  
 25 STALRCSSSEGAPKPVYNWVRLGTFPTSPGSMVQDEVSGQLILTNLSLTSSG  
 TYRCVATNQMGASCELTLSVTEPSQGRVAEL (SEQ ID NO: 97).

Polynucleotides encoding these polypeptides are also provided.

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 246-262 of the amino acid sequence referenced  
 30 in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 263-327 of this protein has also been determined. Based upon these characteristics, it is

believed that the protein product of this gene shares structural features to type Ia membrane proteins.

This gene is expressed primarily in colon and colon cancer.

Therefore, polynucleotides and polypeptides of the invention are useful as  
5 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, proliferative disorders, particularly of the immune or gastrointestinal system, such as colon cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential  
10 identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system or gastrointestinal system, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g., immune, gastrointesinal, digestive, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, bile, serum, plasma, urine,  
15 synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise immunogenic  
20 epitopes shown in SEQ ID NO: 60 as residues: Pro-64 to Glu-70, Pro-84 to Lys-90, His-112 to Gly-117, Asn-124 to Tyr-130, Pro-148 to Gln-155, Ser-167 to Pro-173, Gln-267 to Gly-279. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in colon and colon cancer tissues combined with the homology to the A33, and to a lesser extent, to the CTX protein indicates that  
25 polynucleotides and polypeptides corresponding to this gene are useful for diagnosis, treatment and/or detection of tumors, especially of the intestine, such as, carcinoid tumors, lymphomas, cancer of the colon and cancer of the rectum, as well as cancers in other tissues where expression has been indicated. Similarly, expression within cellular sources marked by proliferating cells indicates that this protein may play a  
30 role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Embryonic development also

involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus, this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer therapy.

Moreover, the expression in the colon tissue may indicate the gene or its products can be used in the disorders of the colon, including inflammatory disorders such as, diverticular colon disease (DCD), inflammatory colonic disease, Crohn's Disease (CD), non-inflammatory bowel disease (non-IBD) colonic inflammation; ulcerative disorders such as, ulcerative colitis (UC), amebic colitis, eosinophilic colitis; noncancerous tumors, such as, polyps in the colon, adenomas, leiomyomas, lipomas, and angiomas. Further, the colon specific expression and cell surface localization indicates that this gene is a good target for antagonists, particularly small molecules or antibodies, which inhibit the biological function of the protein encoded by this gene. Accordingly, preferred are antibodies and or small molecules which specifically bind an extracellular portion of the translation product of this gene. The extracellular regions can be ascertained from the information regarding the transmembrane domains as set out above.

Also provided is a kit for detecting colon cancer. Such a kit comprises in one embodiment an antibody specific for the translation product of this gene bound to a solid support. Also provided is a method of detecting colon cancer in an individual which comprises a step of contacting an antibody specific for the translation product of this gene to a bodily fluid from the individual, preferably serum, and ascertaining whether antibody binds to an antigen found in the bodily fluid. Preferably the antibody is bound to a solid support and the bodily fluid is serum. The above embodiments, as well as other treatments and diagnostic tests (kits and methods), are more particularly described elsewhere herein. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly

available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:23 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
5 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1116 of SEQ ID NO:23, b is an integer of 15 to 1130, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:23, and where b is greater than or equal to a + 14.

#### 10 **FEATURES OF PROTEIN ENCODED BY GENE NO: 14**

The gene encoding the disclosed cDNA is believed to reside on chromosome 7. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 7.

15 This gene is expressed primarily in thymus, uterine cancer, and hepatoma.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, disorders of the immune system, particularly cancer or tumors of the reproductive tract or hepatic system. Similarly, polypeptides and antibodies directed  
20 to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types  
25 (e.g. immune, hepatic, reproductive, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, amniotic fluid, bile, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

30 Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 61 as residues: Val-36 to Leu-43. Polynucleotides

encoding said polypeptides are also provided.

The tissue distribution in thymus indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the

5 "Immune Activity" and "infectious disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Expression of this gene product in thymus indicates a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen

10 presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses).

Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such

15 as AIDS, leukemia, rheumatoid arthritis, granulomatous Disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus

20 erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's Disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Similarly, expression within cellular sources marked by proliferating cells

25 indicates that this protein may play a role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic development also involves decisions involving cell differentiation and/or apoptosis in pattern formation. Thus this protein may also be involved in apoptosis or tissue differentiation and could again be useful in cancer

30 therapy. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify



agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly  
5 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:24 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
10 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1424 of SEQ ID NO:24, b is an integer of 15 to 1438, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:24, and where b is greater than or equal to a + 14.

## 15 FEATURES OF PROTEIN ENCODED BY GENE NO: 15

The gene encoding the disclosed cDNA is believed to reside on chromosome 7. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 7.

This gene is expressed primarily in keratinocytes and leukocytes.

20 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, disorders affecting the immune system, especially lymphomas or autoimmune conditions, in addition to integumentary disorders. Similarly,  
25 polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g.integumentary, immune, hematopoietic, and  
30 cancerous and wounded tissues) or bodily fluids (e.g.lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an

individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in keratinocytes and leukocytes indicates that

5 polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of disorders affecting the skin and the immune system, especially lymphomas. Representative uses are described in the "Biological Activity", "Hyperproliferative Disorders", "infectious disease", and "Regeneration" sections below, in Example 11, 19, and 20, and elsewhere herein. Briefly, the protein product

10 of this gene would also be useful for the treatment, diagnosis, and/or prevention of various skin disorders including congenital disorders (i.e. nevi, moles, freckles, Mongolian spots, hemangiomas, port-wine syndrome), integumentary tumors (i.e. keratoses, Bowen's Disease, basal cell carcinoma, squamous cell carcinoma, malignant melanoma, Paget's Disease, mycosis fungoides, and Kaposi's sarcoma),

15 injuries and inflammation of the skin (i.e. wounds, rashes, prickly heat disorder, psoriasis, dermatitis), atherosclerosis, urticaria, eczema, photosensitivity, autoimmune disorders (i.e. lupus erythematosus, vitiligo, dermatomyositis, morphea, scleroderma, pemphigoid, and pemphigus), keloids, striae, erythema, petechiae, purpura, and xanthelasma. In addition, such disorders may predispose increased susceptibility to

20 viral and bacterial infections of the skin (i.e. cold sores, warts, chickenpox, molluscum contagiosum, herpes zoster, boils, cellulitis, erysipelas, impetigo, tinea, athletes foot, and ringworm).

Moreover, the protein product of this gene may also be useful for the treatment or diagnosis of various connective tissue disorders such as arthritis, trauma,

25 tendonitis, chondromalacia and inflammation, autoimmune disorders such as rheumatoid arthritis, lupus, scleroderma, and dermatomyositis as well as dwarfism, spinal deformation, and specific joint abnormalities as well as chondrodysplasias (ie. spondyloepiphyseal dysplasia congenita, familial osteoarthritis, Atelosteogenesis type II, metaphyseal chondrodysplasia type Schmid). Alternatively, this gene product is

30 involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune

responses).

Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous Disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's Disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:25 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 902 of SEQ ID NO:25, b is an integer of 15 to 916, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:25, and where b is greater than or equal to a + 14.

**FEATURES OF PROTEIN ENCODED BY GENE NO: 16**

The translation product of this gene shares sequence homology with several genes which are thought to be important in autosomal dominant polycystic kidney disease type II (See Genbank Accession No.gi|2558835 (AF014010)).

5           The polypeptide of this gene has been determined to have six transmembrane domains at about amino acid positions 230 - 246, 286 - 302, 317 - 333, 344 - 360, 362 - 378, and 432 - 448 of the amino acid sequence referenced in Table 1 for this gene. Based upon these characteristics, it is believed that the protein product of this gene shares structural features to type IIIa membrane proteins.

10           Included in this invention as preferred domains is a lipase, serine active site domain, which was identified using the ProSite analysis tool (Swiss Institute of Bioinformatics). Triglyceride lipases (EC 3.1.1.3) [1] are lipolytic enzymes that hydrolyzes the ester bond of triglycerides. Lipases are widely distributed in animals, plants and prokaryotes. In higher vertebrates there are at least three tissue- specific

15           isozymes: pancreatic, hepatic, and gastric/lingual. These three types of lipases are closely related to each other as well as to lipoprotein lipase (EC 3.1.1.34) [2], which hydrolyzes triglycerides of chylomicrons and very low density lipoproteins (VLDL). The most conserved region in all these proteins is centered around a serine residue which has been shown [3] to participate, with an histidine and an aspartic acid

20           residue, to a charge relay system. Such a region is also present in lipases of prokaryotic origin and in lecithin-cholesterol acyltransferase (EC 2.3.1.43) (LCAT) [4], which catalyzes fatty acid transfer between phosphatidylcholine and cholesterol. The concensus pattern is as follows:[LIV]-x-[LIVFY]-[LIVMST]-G-[HYWV]-S-x-G-[GSTAC], S is the active site residue.

25           Preferred polypeptides of the invention comprise the following amino acid sequence: LFLLGYS DGA (SEQ ID NO: 98). Polynucleotides encoding these polypeptides are also provided.

          Further preferred are polypeptides comprising the lipases, serine active site domain of the sequence referenced in Table for this gene, and at least 5, 10, 15, 20,

30           25, 30, 50, or 75 additional contiguous amino acid residues of this referenced sequence. The additional contiguous amino acid residues is N-terminal or C- terminal

to the lipases, serine active site domain. Alternatively, the additional contiguous amino acid residues is both N-terminal and C-terminal to the lipases, serine active site domain, wherein the total N- and C-terminal contiguous amino acid residues equal the specified number. The above preferred polypeptide domain is characteristic of a signature specific to lipase proteins. Based on the sequence similarity, the translation product of this gene is expected to share at least some biological activities with lipases proteins, in addition to other proteins involved in fatty acid metabolism. Such activities are known in the art, some of which are described elsewhere herein. The following publications were referenced above and are hereby incorporated herein by reference: [ 1] Chapus C., Rovey M., Sarda L., Verger R., *Biochimie* 70:1223-1234(1988); [ 2] Persson B., Bengtsson-Olivecrona G., Enerback S., Olivecrona T., Joernvall H., *Eur. J. Biochem.* 179:39-45(1989); [ 3] Blow D., *Nature* 343:694-695(1990); [ 4] McLean J., Fielding C., Drayna D., Dieplinger H., Baer B., Kohr W., Henzel W., Lawn R., *Proc. Natl. Acad. Sci. U.S.A.* 83:2335-2339(1986); and [ 5] Baker M.E., *Biochem. J.* 255:1057-1060(1988).

This gene is expressed most cells in the immune system, and to a lesser extent, in liver, spleen, and placenta.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, renal disorders, particularly autosomal dominant polycystic kidney disease, in addition to a variety of immune, hepatic, or developmental disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and detoxification systems, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g. renal, immune, hepatic, hematopoietic, developmental, reproductive, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, amniotic fluid, bile, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e.,

the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 63 as residues: Gly-83 to Ser-92, Tyr-100 to Phe-113, Asp-127 to Pro-134. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution and homology to genes involved with autosomal dominant polycystic kidney disease type II indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of autosomal dominant polycystic kidney disease type II and other disorders affecting cells of the immune system. Similarly, the homology also indicates that this gene or gene product could be used in the treatment and/or detection of other kidney diseases including renal failure, nephritis, renal tubular acidosis, proteinuria, pyuria, edema, pyelonephritis, hydronephritis, nephrotic syndrome, crush syndrome, glomerulonephritis, hematuria, renal colic and kidney stones, in addition to Wilms Tumor Disease, and congenital kidney abnormalities such as horseshoe kidney, polycystic kidney, and Falconi's syndrome. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Moreover, representative uses are described in the "Immune Activity" and "infectious disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses).

Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous Disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as

T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's Disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:26 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2080 of SEQ ID NO:26, b is an integer of 15 to 2094, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:26, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 17**

The gene encoding the disclosed cDNA is believed to reside on chromosome 4. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 4.

This gene is expressed primarily in brain, kidney and T cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, neural or immune disorders, particularly neurodegenerative disorders such as Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, or cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the brain, central nervous system and the immune system, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g. neural, endocrine, renal, immune, hematopoietic, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in neural tissue indicates that polynucleotides and polypeptides corresponding to this gene are useful for the detection/treatment of neurodegenerative disease states, behavioural disorders, or inflammatory conditions.

Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and preception. In addition, Elevated expression of this gene product in regions of the brain indicates that it plays a role in normal neural function.

Potentially, this gene product is involved in synapse formation,



neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Moreover, the gene or gene product may also play a role in the treatment and/or detection of developmental disorders associated with the developing embryo, sexually-linked disorders, or disorders of the cardiovascular system. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:27 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2062 of SEQ ID NO:27, b is an integer of 15 to 2076, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:27, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 18

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:

LNNSPLYENTTFYLSTHQVMAIWVVFYIWLLL VFCEHSCISFRVDVCIHFSCN  
KFYLGVELLDHMAALLTLWGTARLLFKVSAPCSLSSAVYDGSVSSQPHQYLF  
SVCRWGLLEHHHHHSFTYYLWLLQYS (SEQ ID NO: 99). Polynucleotides encoding these polypeptides are also provided.

This gene is expressed primarily in liver and adipose tissue.

Therefore, polynucleotides and polypeptides of the invention are useful as

reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, disorders affecting the liver and adipose tissue, including cancer, cirrhosis, and obesity. Similarly, polypeptides and antibodies directed to these

5 polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the hematopoietic and immune systems, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g. hepatic, and cancerous and wounded tissues) or bodily fluids  
10 (e.g. lymph, bile, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise immunogenic  
15 epitopes shown in SEQ ID NO: 65 as residues: Ser-76 to Gln-83. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for the detection and treatment of liver disorders and cancers (e.g. hepatoblastoma, jaundice, hepatitis, liver metabolic diseases and  
20 conditions that are attributable to the differentiation of hepatocyte progenitor cells). Representative uses are described in the "Hyperproliferative Disorders", "infectious disease", and "Binding Activity" sections below, in Example 11, and 27, and elsewhere herein. Briefly, the the protein may show utility in developmental abnormalities, fetal deficiencies, pre-natal disorders and various wound-healing  
25 models and/or tissue trauma. Molecules of the present invention is involved in regulating the growth of Schwann cells and other neural cells. Nucleic acids of the present invention are useful as probes for detecting traumatic and pathological changes in the central and peripheral nervous systems.

The protein is useful in detecting, treating, and/or preventing diseases and/or  
30 disorders which occur secondary to deficiencies in fatty acid metabolism, particularly aberrations in myelin sheath maintenance and regeneration. Molecules of

the present invention are also useful as modulators of the interaction between Schwann cells and other neural cells and the extracellular matrix and is therefore useful for the therapeutic intervention in nerve damage primarily by facilitating regeneration of damaged axons and regenerating nerve cells in damaged nervous system tissues. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:28 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1364 of SEQ ID NO:28, b is an integer of 15 to 1378, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:28, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 19**

Preferred polypeptides of the invention comprise the following amino acid sequence: LLNKTTFYLPMARQVFFQLSPIHPVPSNLSMGWNLTG (SEQ ID NO: 100). Polynucleotides encoding these polypeptides are also provided.

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:

LLNKTTFYLPMARQVFFQLSPIHPVPSNLSMGWNLTGMTFGIVVDLTPVFVL  
VLFLPAFLFLSLPSWSLPRDPHTVKYGLEDCMNAS (SEQ ID NO: 101).

Polynucleotides encoding these polypeptides are also provided.

This gene is expressed primarily in fetal lung, placenta, and to a lesser extent in brain, melanocytes and endothelial cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a

5 biological sample and for diagnosis of diseases and conditions which include, but are not limited to, monitoring of fetal lung development and diseases of the lung

including ARDS, in addition to reproductive, neural or immune disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing

immunological probes for differential identification of the tissue(s) or cell type(s). For

10 a number of disorders of the above tissues or cells, particularly of the respiratory

system, expression of this gene at significantly higher or lower levels is routinely

detected in certain tissues or cell types (e.g. pulmonary tissues, developmental, neural, endothelial, vascular, and cancerous and wounded tissues) or bodily fluids

(e.g. lymph, amniotic fluid, pulmonary surfactant or sputum, serum, plasma, urine,

15 synovial fluid and spinal fluid) or another tissue or cell sample taken from an

individual having such a disorder, relative to the standard gene expression level, i.e.,

the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution in fetal lung and placenta indicates that polynucleotides

20 and polypeptides corresponding to this gene are useful for diagnosing and treating developmental defects of the lung. Similarly, expression within fetal tissue and other

cellular sources marked by proliferating cells indicates that this protein may play a

role in the regulation of cellular division, and may show utility in the diagnosis and treatment of cancer and other proliferative disorders. Similarly, embryonic

25 development also involves decisions involving cell differentiation and/or apoptosis in

pattern formation. Thus this protein may also be involved in apoptosis or tissue

differentiation and could again be useful in cancer therapy. In addition, the expression

within fetal lung, brain, placenta, and endothelial cells and tissues, combined,

indicates that the protein is useful in the detection, treatment, and/or prevention of a

30 variety of vascular disorders and conditions, which include, but are not limited to

microvascular disease, vascular leak syndrome, aneurysm, stroke, embolism,

thrombosis, coronary artery disease, arteriosclerosis, and/or atherosclerosis.

Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement.

- 5 Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:29 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1951 of SEQ ID NO:29, b is an integer of 15 to 1965, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:29, and where b is greater than or equal to a + 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 20

The translation product of this gene shares sequence homology with a C. elegans protein (See Genbank Accession No.gi|746495).

Preferred polypeptides of the invention comprise the following amino acid sequence:

NSARAAAEGRGLRTPGFRGGGVLYWDAGAAGTGSNHALGANVELWI (SEQ ID NO: 102). Polynucleotides encoding these polypeptides are also provided.

25 In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:

NSARAAAEGRGLRTPGFRGGGVLYWDAGAAGTGSNHALGANVELWIMLL  
30 QVVREGKFSGFLTSCSLLLPRAAQILAAEAGLPSSRSFMGFAAPFTNKRKAYS  
ERRIMGYSMQEMYEVVSNVQEYREFVPWCKKSLVSSRKGHLKAQLEVGFP

PVMERYTSAVSMVKPHMVKA VCTDGKLFNHLETIWRFSPGIPAYPRTCTVDF  
SISFEFRSLLHSQ LATMFFDEVVKQNVAAFERRAATKFGPETAI PRELMFHEV  
HQT (SEQ ID NO: 103). Polynucleotides encoding these polypeptides are also provided.

5 The gene encoding the disclosed cDNA is believed to reside on chromosome 20. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 20.

This gene is expressed primarily in melanocytes, fetal liver and spleen and lung, and to a lesser extent, in hepatocellular tumor, spleen and infant brain.

10 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immune, hepatic or developmental disorders, particularly cancers such as melanoma and hepatocellular tumors. Similarly, polypeptides and antibodies  
15 directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the skin, lung and liver, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g. integumentary tissues, developmental, immune, hematopoietic,  
20 pulmonary, neural tissues, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, amniotic fluid, pulmonary surfactant or sputum, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the  
25 disorder.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 67 as residues: Thr-49 to Arg-58, Val-75 to Phe-81. Polynucleotides encoding said polypeptides are also provided.

30 The tissue distribution in melanocytes, fetal liver, and hepatocellular cells and tissues indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosing and treating tumors including melanoma and hepatocellular

tumors. Representative uses are described in the "Hyperproliferative Disorders" and "Regeneration" sections below and elsewhere herein. Thus, aberrant expression of this gene product in tissues - particularly adult tissues - may correlate with patterns of abnormal cellular proliferation, such as found in various cancers. Because of potential roles in proliferation and differentiation, this gene product may have applications in the adult for tissue regeneration and the treatment of cancers. It may also act as a morphogen to control cell and tissue type specification. Similarly, expression of this gene product in immune tissues indicates a role in the regulation of the proliferation; survival; differentiation; and/or activation of potentially all hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes that may also suggest a usefulness in the treatment of cancer (e.g. by boosting immune responses).

Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also used as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous Disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's Disease, scleroderma and tissues. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are

related to SEQ ID NO:30 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
 5 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1459 of SEQ ID NO:30, b is an integer of 15 to 1473, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:30, and where b is greater than or equal to a + 14.

# 10 FEATURES OF PROTEIN ENCODED BY GENE NO: 21

The translation product of this gene shares sequence homology with dehydrogenases which are thought to be important in modifying the structure of small molecules, particularly during biosynthesis reactions (See Genbank Accession No.gi|1125836).

15 Preferred polypeptides of the invention comprise the following amino acid sequence: R W I F F Q K C R P I L I K F V I N H W G G Q A P W I R S A F G D T (SEQ ID NO: 104).

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the  
 20 following amino acid sequence:

R W I F F Q K C R P I L I K F V I N H W G G Q A P W I R S A F G D T M G V M A M L M L P L L L L G I S G L  
 L F I Y Q E V S R L W S K S A V Q N K V V V I T D A I S G L G K E C A R V F H T G G A R L V L C G K N W  
 E R L E N L Y D A L I S V A D P S K T F T P K L V L L D L S D I S C V P D V A K E V L D C Y G C V D I L I N  
 N A S V K V K G P A H K I S L E L D K K I M D A N Y F G P I T L T K A L L P N M I S R R T G Q I V L V N N  
 25 I Q G K F G I P F R T T Y A A S K H A A L G F F D C L R A E V E E Y D V V I S T V S P T F I R S Y H V Y P E  
 Q G N W E A S I W K F F F R K L T Y G V H P V X V A E E V M R T V R R K K Q E V F M A N P I P K A A V  
 Y V R T F F P E F F F A V V A C G V K E K L N V P E E G (SEQ ID NO: 105). Polynucleotides  
 encoding these polypeptides are also provided. Polynucleotides encoding these  
 polypeptides are also provided. Particularly preferred are polypeptide variants of the  
 30 protein encoded by this gene which retain enzymatic activity, preferably  
 dehydrogenase activity. Such activities is assayed according to art known techniques



and as described elsewhere herein.

Included in this invention as preferred domains are short-chain dehydrogenases/reductases family signature domains, which were identified using the ProSite analysis tool (Swiss Institute of Bioinformatics). The short-chain dehydrogenases/reductases family (SDR) [1] is a very large family of enzymes, most of which are known to be NAD- or NADP-dependent oxidoreductases. As the first member of this family to be characterized was *Drosophila* alcohol dehydrogenase, this family used to be called [2,3,4] 'insect-type', or 'short-chain' alcohol dehydrogenases. Most member of this family are proteins of about 250 to 300 amino acid residues. The concensus pattern is as follows: [LIVSPADNK]-x(12)-Y-[PSTAGNCV]-[STAGNQCIVM]-[STAGC]-K-{PC}-[SAGFYR]-[LIVMSTAGD]-x(2)-[LIVMFYW]-x(3)-[LIVMFYWGAPTHQ]-[GSACQRHM], Y is an active site residue.

Preferred polypeptides of the invention comprise the following amino acid sequence: NIQGKFGIPFRTTYAASKHAALGFFDCLR (SEQ ID NO: 106). Polynucleotides encoding these polypeptides are also provided.

Further preferred are polypeptides comprising the short-chain dehydrogenases/reductases family signature domain of the sequence referenced in Table for this gene, and at least 5, 10, 15, 20, 25, 30, 50, or 75 additional contiguous amino acid residues of this referenced sequence. The additional contiguous amino acid residues is N-terminal or C- terminal to the short-chain dehydrogenases/reductases family signature domain. Alternatively, the additional contiguous amino acid residues is both N-terminal and C-terminal to the short-chain dehydrogenases/reductases family signature domain, wherein the total N- and C-terminal contiguous amino acid residues equal the specified number. The above preferred polypeptide domain is characteristic of a signature specific to dehydrogenase proteins. Based on the sequence similarity, the translation product of this gene is expected to share at least some biological activities with dehydrogenase proteins. Such activities are known in the art, some of which are described elsewhere herein. The following citations were references above and are hereby incorporated herein by reference: [ 1] Joernvall H., Persson B., Krook M., Atrian S., Gonzalez-

Duarte R., Jeffery J., Ghosh D., Biochemistry 34:6003-6013(1995); [ 2] Villarroya A., Juan E., Egestad B., Joernvall H., Eur. J. Biochem. 180:191-197(1989), [ 3] Persson B., Krook M., Joernvall H., Eur. J. Biochem. 200:537-543(1991); and [ 4] Neidle E.L., Hartnett C., Ornston N.L., Bairoch A., Rekik M., Harayama S., Eur. J. Biochem. 204:113-120(1992).

This gene is expressed primarily in heart.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, abnormalities of the cardiovascular system such as ischemic heart disease. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the cardiovascular system, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g. vascular, cardiopulmonary, and cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 68 as residues: Lys-69 to Glu-75, Asp-86 to Thr-92. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in heart and homology to dehydrogenases indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosing, monitoring, or treating diseases of the cardiovascular system. Alternatively, the protein is useful in the detection, treatment, and/or prevention of vascular conditions, which include, but are not limited to, microvascular disease, vascular leak syndrome, aneurysm, stroke, atherosclerosis, arteriosclerosis, or embolism. For example, this gene product may represent a soluble factor produced by smooth muscle that regulates the innervation of organs or regulates the survival of

neighboring neurons. Likewise, it is involved in controlling the digestive process, and such actions as peristalsis. Similarly, it is involved in controlling the vasculature in areas where smooth muscle surrounds the endothelium of blood vessels. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as  
5 tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly  
10 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:31 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or  
15 more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1143 of SEQ ID NO:31, b is an integer of 15 to 1157, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:31, and where b is greater than or equal to a + 14.

## 20 **FEATURES OF PROTEIN ENCODED BY GENE NO: 22**

The translation product of this gene shares sequence homology with the human TMPRSS2 gene product, which is a novel serine protease with transmembrane, LDLRA, and SRCR domains (see Genbank protein accession no. AAC51784 and Genomics 44 (3), 309-320 (1997); all references and information  
25 available through this accession are hereby incorporated by reference herein.)

Included in this invention as preferred domains are trypsin family serine protease domains (containing histidine or serine active sites), which were identified using the ProSite analysis tool (Swiss Institute of Bioinformatics). The catalytic activity of the serine proteases from the trypsin family is provided by a charge relay  
30 system involving an aspartic acid residue hydrogen-bonded to a histidine, which itself is hydrogen-bonded to a serine. The sequences in the vicinity of the active site serine

and histidine residues are well conserved in this family of proteases (see Brenner S.; Nature 334:528-530 (1988)). A partial list of proteases known to belong to the trypsin family include: acrosin; blood coagulation factors VII, IX, X, XI and XII; thrombin; plasminogen; chymotrypsins; complement components C1r, C1s, C2; complement factors B, D and I; cytotoxic cell proteases; plasminogen activators (urokinase-type, and tissue-type); trypsin I, II, III, and IV; and collagenases. The consensus pattern for domains with histidine as the active residue is as follows: [LIVM]-[ST]-A-[STAG]-H-C. The consensus pattern for domains with serine as the active residue is: [DNSTAGC]-[GSTAPIMVQH]-x(2)-G-[DE]-S-G-[GS]-[SAPHV]-[LIVMFYWH]-[LIVMFYSTANQH].

Preferred polypeptides of the invention comprise the following amino acid sequences: TCGGSVLAPRWVVTAAHCMHSFRLARLSSW (SEQ ID NO: 109); and CAGYLDGRADACQGDSGGPLVCPDGDWRL (SEQ ID NO: 110).

Polynucleotides encoding these polypeptides are also provided.

Further preferred are polypeptides comprising the VTAAHC and DACQGDSGGPLV domains of the sequence referenced in Table 1 for this gene, and at least 5, 10, 15, 20, 25, 30, 50, or 75 additional contiguous amino acid residues of this referenced sequence. The additional contiguous amino acid residues is N-terminal or C-terminal to the VTAAHC and DACQGDSGGPLV domains. Alternatively, the additional contiguous amino acid residues is both N-terminal and C-terminal to the VTAAHC and DACQGDSGGPLV domains, wherein the total N- and C-terminal contiguous amino acid residues equal the specified number. The above preferred polypeptide domains are characteristic of signatures specific to serine proteases. Based on the sequence similarity, the translation product of this gene is expected to share at least some biological activities with serine protease proteins (see Brenner S.; Nature 334:528-530 (1988) and Rawlings N.D., Barrett A.J.; Meth. Enzymol. 244:19-61 (1994); all references and information available through these publications are hereby incorporated by reference herein). Such activities are known in the art, some of which are described elsewhere herein. Polynucleotides encoding these polypeptides are also provided.

In another embodiment, polypeptides comprising the amino acid sequence of

the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequences:

DPRVRACLSTQRDISSRAITQPQRRNPNTFCCCFSTILWVLDWLSQACCPAA  
5 SLPVSFSQAVCWRSMRRGCAVLGALGLLAGAGVGSWLLVLYLCPAASQPIS  
GTLQDEEITLSCSEASAEELLPALPKTVSFRINSEDFLLEAQVRDQPRWLLVC  
HEGWSPALGLQICWSLGHRLRTHHKGVNLTDIKLNSSQEFAQLSPRLGGFLEE  
AWQPRNNCTSGQVVSLRCSECGARPLASRIVGGQSVAPGRWPWQASVALGF  
RHTCGGSVLAPRWVVTAAHCMHSFRLARLSSWRVHAGLVSHSAVVRPHQGA  
10 LVERIIPHPLYSAQNHDYDVALLRLQTALNFSDTVGAVCLPAKEQHFPKGSRC  
WVSGWGHTHPSHTYSSDMLQDTVVPLFSTQLCNSSCVYSGALTTPRMLCAGY  
LDGRADACQGDSGGPLVCPDGDWRLVGVVSWGRGCAEPNHPGVYAKVAE  
FLDWIHDTAQDSLL (SEQ ID NO: 107); and  
DPRVRACLSTQRDISSRAITQPQRRNPNTFCCCFSTILWVLDWLSQACCPAA  
15 SLPVSFSQAVCWRS (SEQ ID NO: 108). Polynucleotides encoding these  
polypeptides are also provided.

The gene encoding the disclosed cDNA is believed to reside on chromosome 11. Accordingly, polynucleotides related to this invention are useful as a marker in linkage analysis for chromosome 11.

20 This gene is expressed primarily in brain and, to a lesser extent, in fetal heart, spleen, liver.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are  
25 not limited to, diagnosis and treatment of hypertension, cardiac hypertrophy, arthritis, inflammatory disorders, blood clotting disorders, hepatic and splenic disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the  
30 central nervous system, cardiac system, and hepatic system, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types

(e.g., cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 69 as residues: Ala-145 to Ser-154, Ala-258 to Tyr-263, Ala-287 to Arg-297, Thr-306 to Met-316. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution and homology to a serine protease indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of brain diseases such as brain tumor, Alzheimers' disease, amnesia, and schizophrenia. Moreover, the tissue distribution in fetal liver indicates polynucleotides and polypeptides corresponding to this gene are useful for the detection and treatment of hepatic and immune disorders. Briefly, the protein can be used for the detection, treatment, and/or prevention of hepatoblastoma, jaundice, hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells. Tissue distribution in the spleen indicates polynucleotides and polypeptides corresponding to this gene are useful for regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages. Additionally, expression in the fetus would suggest a useful role for the protein product in developmental abnormalities, fetal deficiencies, pre-natal disorders and various wound-healing models and/or tissue trauma. Protein, as well as, antibodies directed against the protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues and in addition hypertension, cardiac hypertrophy, arthritis, inflammatory disorders and blood clotting disorders.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:32 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2176 of SEQ ID NO:32, b is an integer of 15 to 2190, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:32, and where b is greater than or equal to a + 14.

### FEATURES OF PROTEIN ENCODED BY GENE NO: 23

The translation product of this gene shares sequence homology with a *C. elegans* gene similar to the DPTI/Kunitz family of inhibitors (see Genbank Accession No. AAC25867.1; all references and information available through this accession are hereby incorporated by reference herein.). This *C. elegans* gene is most similar to tissue factor pathway inhibitor precursor.

Preferred polypeptides of the invention comprise the following amino acid sequence:

CRNSARAFSGLSMVAYSQVLAVFISCAITLAMKIAWIFGLNSVQNITANLS  
VDGSTSGNPIQKWKVIWSL (SEQ ID NO: 111).

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence: CRNSARAFSGLS (SEQ ID NO: 112).

Polynucleotides encoding this polypeptide is also provided.

This gene is expressed primarily in endothelial cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, tissue ischemia, myocardioinfarction. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the circulatory system, expression of this gene at significantly higher or lower levels is routinely detected in

certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution and homology to a *C. elegans* gene that is similar to the DPTI/Kunitz family of inhibitors indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of tissue ischemia as well as myocardioinfarction. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:33 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2497 of SEQ ID NO:33, b is an integer of 15 to 2511, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:33, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 24**

The translation product of this gene shares sequence homology with yeast gene CDC91 (see Genbank Accession No. AAA34487; all references and information available through this accession are hereby incorporated by reference herein.).

Preferred polypeptides of the invention comprise the following amino acid sequence:



MITDALTAIALYFAIQDFNKVVFKKQKLLLELDQYAPDVAELIRTPMEMRYIP  
 LKVALFYLLNPYITLSCVAKSTCAINNTLIAFFILTTIKGSAFLSAIFLALATYQS  
 LYPLTLFVPGLLYLLQRQYIPVKMKSKAFWIFSWEYAMMYVGSLVVIICLSFF  
 LLSSWDFIPAVYGFILSVPDLTPNIGLFWYFFAEMFEHFSLFFVCVFQINVFFYT  
 5 IPLAIKLKEHPIFFMFIQIAVIAIFKSYPTVGDVALYMAFFPVWNHLYRFLRNIF  
 VLTCHIVCSLLFPVLWHLWIYPGNANSNFFYAITLTFNVGQILLISDYFYAFLR  
 REYYLTHGLYLTAKDGTEAMLVLK (SEQ ID NO: 113). Polynucleotides  
 encoding such polypeptides are also provided.

In another embodiment, polypeptides comprising the amino acid sequence of  
 10 the open reading frame upstream of the predicted signal peptide are contemplated by  
 the present invention. Specifically, polypeptides of the invention comprise the  
 following amino acid sequences:

PTRPRAPAPVIMAAPLVLVVAVTVRAALFRSSLAEFISERVEVVSPLSSWK  
 RVVEGLSLLGLGSISVFWSSISWKLHSL (SEQ ID NO: 114); and PTRPRAPAPVI  
 15 (SEQ ID NO: 115). Polynucleotides encoding these polypeptides are also provided.

In another embodiment, polypeptides comprising the amino acid sequence of  
 the open reading frame upstream of the predicted signal peptide are contemplated by  
 the present invention. Specifically, polypeptides of the invention comprise the  
 following amino acid sequences:

20 IYLFHFLIDYAEVFMITDALTAIALYFAIQDFNKVVFKKQKLLLELDQYAPD  
 VAELIRTPMEMRYIPLKVALFYLLNPYITLSCVAKSTCAINNTLIAFFILTTIKGS  
 AFLSAIFLALATYQSLYPLTLFVPGLLYLLQRQYIPVKMKSKAFWIFSWEYAM  
 MYVGSLVVIICLSFFLLSSWDFIPAVYGFILSVPDLTPNIGLFWYFFAEMFEHFS  
 LFFVCVFQINVFFYTIPLAIKLKEHPIFFMFIQIAVIAIFKSYPTVGDVALYMAFF  
 25 PVWNHLYRFLRNIFVLTCHIVCSLLFPVLWHLWIYPGMPTLISFMPH (SEQ ID  
 NO:116); IYLFHFLIDYAEVFM (SEQ ID NO: 117);

MITDALTAIALYFAIQDFNKVVFKKQKLLLELDQYAPDVAELIRTPMEMRYIP  
 LKVALFYLLNPYITLSCVAKSTCAINNTLIAFFILTTIKGSAFLSAIFLALATYQS  
 LYPLTLFVPGLLYLLQRQYIPVKMKSKAFWIFSWEYAMMYVGSLVVIICLSFF  
 30 LLSSWDFIPAVYGFILSVPDLTPNIGLFWYFFAEMFEHFSLFFVCVFQINVFFYT  
 IPLAIKLKEHPIFFMFIQIAVIAIFKSYPTVGDVALYMAFFPVWNHLYRFLRNIF

VLTCIIIVCSLLFPVLWHLWIYPGMPTLISFMPSH (SEQ ID NO: 118);  
MITDALTAIALYFAIQDFNKVVFKKQKLLLELDQY (SEQ ID NO: 119);  
APDVAELIRTPMEMRYIPLKVALFYLLNPYTILSC (SEQ ID NO: 120);  
VAKSTCAINNTLIAFFILTTIKGSAFLSAIFLALA (SEQ ID NO: 121);  
5 TYQSLYPLTLFVPGLLYLLQRQYIPVKMKSKAFWI (SEQ ID NO: 122);  
FSWEYAMMYVGSLVVIICLSFFLLSSWDFIPAVYG (SEQ ID NO: 123);  
FILSVPDLTPNIGLFWYFFAEMFEHFSLFFVCVFQ (SEQ ID NO: 124);  
INVFFYTIPLAIKLKEHPIFFMFIQIAVIAIFKSY (SEQ ID NO: 125);  
PTVGDVALYMAFFPVWNHLYRFLRNIFVLTCIIIV (SEQ ID NO: 126);  
10 CSLLFPVLWHLWIYPGMPTLISFMPSH (SEQ ID NO: 127). The polypeptide  
IYLFHFLIDYAEVFMITDALTAIALYFAIQDFNKVVFKKQKLLLELDQYAPD  
VAELIRTPMEMRYIPLKVALFYLLNPYTILSCVAKSTCAINNTLIAFFILTTIKGS  
AFLSAIFLALATYQSLYPLTLFVPGLLYLLQRQYIPVKMKSKAFWIFSWEYAM  
MYVGSLVVIICLSFFLLSSWDFIPAVYGFILSVPDLTPNIGLFWYFFAEMFEHFS  
15 LFFVCVFQINVFFYTIPLAIKLKEHPIFFMFIQIAVIAIFKSYPTVGDVALYMAFF  
PVWNHLYRFLRNIFVLTCIIIVCSLLFPVLWHLWIYPGMPTLISFMPSH (SEQ ID  
NO:116) has been determined to have transmembrane domains at about amino acid  
positions 11-27, 68-84, 93-109, 160-176, 214-230, 241-257, and 283-299. Based  
upon these characteristics, it is believed that the protein product of this gene is a seven  
20 transmembrane spanning protein.

This gene is expressed primarily in Jurkat cells and synovocytes, and to a lesser extent in a wide variety of other human tissues.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a  
25 biological sample and for diagnosis of diseases and conditions which include, but are not limited to, rheumatoid arthritis. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at  
30 significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum,

plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

5           The tissue distribution and homology to yeast CDC91 gene product indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of rheumatoid arthritis. Moreover, the expression of this gene product in synovium would suggest a role in the detection and treatment of disorders and conditions affecting the skeletal system, in particular osteoporosis as well as  
10 disorders afflicting connective tissues (e.g. trauma, tendonitis, chondromalacia and inflammation), such as in the diagnosis or treatment of various autoimmune disorders such as lupus, scleroderma, and dermatomyositis as well as dwarfism, spinal deformation, and specific joint abnormalities as well as chondrodysplasias (ie. spondyloepiphyseal dysplasia congenita, familial osteoarthritis, Atelosteogenesis type  
15 II, metaphyseal chondrodysplasia type Schmid). Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are  
20 related to SEQ ID NO:34 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general  
25 formula of a-b, where a is any integer between 1 to 1670 of SEQ ID NO:34, b is an integer of 15 to 1684, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:34, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 25**

30           Preferred polypeptides of this invention comprise the following amino acid sequence:

EPTRGSAMAEQTYSWAYSLVDSSQVSTFLISILLIVYGSFRSLNMDFENQDKE  
 KDSNSSSGSFNGNSTNNSIQTIDSTQALFLPIGASVSLVMFFFFDSVQVVFTIC  
 TAVLATIAFAFLLPMCQYLTRPCSPQNKISFGCCGRFTAAELLSFSLSVMLVL  
 IWVLTGHWLLMDALAMGXCVMIAFVRLPSLKVSCLLLSGLLIYDVFWVFFS  
 5 AYIFNSNVMVKVATQPADNPLDVLSRKLHLGPNVGRDVPRLSLPGKLVFPSS  
 TGSFHSMLGIGDIVMPGLLLCFVLRDNYKKQASGDSCGAPGPANISGRMQK  
 VSYFHCTLIGYFVGLLTATVASRIHRAAQPALLYLVPFTLLPLLTMAYLKGD  
 RRMWSEPFHSKSSSSRFLEV (SEQ ID NO: 128). This protein is believed to

contain multiple transmembrane domains, roughly located at residues 81-97, 106-122,  
 10 151-167, 172-188, 197-213, 274-290. Thus, the extracellular portions of the  
 polypeptide are believed to be useful as antigens. Thus, polypeptides comprising one,  
 or more, of the following amino acid sequences are preferred: residues 41-82, 123-  
 150 and 214-273 all as shown in the amino acid sequence above. Polynucleotides  
 comprising all of the foregoing polypeptides are provided.

15 In another embodiment, polypeptides comprising the amino acid sequence of  
 the open reading frame upstream of the predicted signal peptide are contemplated by  
 the present invention. Specifically, polypeptides of the invention comprise the  
 following amino acid sequence:

EPTRGSAMAEQTYSWAYSLVDSSQVSTFLISILLIVYGSFRSLNMDFENQDKE  
 20 KDSNSSSGSFNGNSTNN SIQTIDSTQALFLPIGASVSLV (SEQ ID NO: 129).  
 Polynucleotides encoding these polypeptides are also provided.

The gene encoding the disclosed cDNA is thought to reside on chromosome  
 12. Accordingly, polynucleotides related to this invention are useful as a marker in  
 linkage analysis for chromosome 12.

25 This gene is expressed primarily in brain, spleen, melanocytes, multiple  
 embryonic tissues, and, to a lesser extent, in a wide variety of other human tissues.  
 Therefore, polynucleotides and polypeptides and polypeptides of the invention are  
 useful as reagents for differential identification of the tissue(s) or cell type(s) present  
 in a biological sample and for diagnosis of diseases and conditions such as brain  
 30 tumors and schizophrenia. Specifically, tissue distribution in the brain indicates  
 polynucleotides and polypeptides corresponding to this gene are useful for the

detection, treatment, and/or prevention of neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to

5 the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive

10 disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function.

Potentially, this gene product is involved in synapse formation,

15 neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. The tissue distribution in spleen indicates polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "infectious disease" sections below, in Example 11, 13, 14, 16, 18, 19,

20 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells.

Moreover, tissue distribution in melanocytes indicates polynucleotides and polypeptides corresponding to this gene are useful for the treatment, diagnosis, and/or

25 prevention of various skin disorders. Representative uses are described in the "Biological Activity", "Hyperproliferative Disorders", "infectious disease", and "Regeneration" sections below, in Example 11, 19, and 20, and elsewhere herein. Briefly, the protein is useful in detecting, treating, and/or preventing congenital disorders (i.e. nevi, moles, freckles, Mongolian spots, hemangiomas, port-wine

30 syndrome), integumentary tumors (i.e. keratoses, Bowen's Disease, basal cell carcinoma, squamous cell carcinoma, malignant melanoma, Paget's Disease, mycosis

fungoides, and Kaposi's sarcoma), injuries and inflammation of the skin (i.e. wounds, rashes, prickly heat disorder, psoriasis, dermatitis), atherosclerosis, urticaria, eczema, photosensitivity, autoimmune disorders (i.e. lupus erythematosus, vitiligo, dermatomyositis, morphea, scleroderma, pemphigoid, and pemphigus), keloids, striae, erythema, petechiae, purpura, and xanthelasma. In addition, such disorders may predispose increased susceptibility to viral and bacterial infections of the skin (i.e. cold sores, warts, chickenpox, molluscum contagiosum, herpes zoster, boils, cellulitis, erysipelas, impetigo, tinea, athlete's foot, and ringworm).

Moreover, the protein product of this gene may also be useful for the treatment or diagnosis of various connective tissue disorders (i.e., arthritis, trauma, tendonitis, chondromalacia and inflammation, etc.), autoimmune disorders (i.e., rheumatoid arthritis, lupus, scleroderma, dermatomyositis, etc.), dwarfism, spinal deformation, joint abnormalities, and chondrodysplasias (i.e. spondyloepiphyseal dysplasia congenita, familial osteoarthritis, Atelosteogenesis type II, metaphyseal chondrodysplasia type Schmid). Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the central nervous system, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g. brain, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 72 as residues: Pro-38 to Ile-45. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for diagnosis and treatment of brain diseases (such as brain tumors and schizophrenia), immune system, and skin disorders. Additionally, protein, as well as, antibodies directed against the protein may show

utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:35 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2369 of SEQ ID NO:35, b is an integer of 15 to 2383, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:35, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 26

When tested against K562 leukemia cell lines, supernatants removed from cells containing this gene activated the ISRE assay. Thus, it is likely that this gene activates leukemia cells through the Jak-STAT signal transduction pathway. Interferon-sensitive response element (ISRE) is a promote element found upstream in many genes which are involved in the Jak-STAT pathway. The Jak-STAT pathway is a large, signal transduction pathway involved in the differentiation and proliferation of cells. Therefore, activation of the Jak-STAT pathway, reflected by the binding of the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells.

The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 26 - 42 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 1 - 25 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features of type II membrane proteins.

This gene is expressed primarily in liver hepatoma.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a

biological sample and for diagnosis of diseases and conditions which include, but are not limited to, hepatoma, as well as other liver disorders and cancers (e.g. hepatoblastoma, hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells). Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the liver, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g. liver, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for detection and treatment of hepatoma, as well as other liver disorders and cancers (e.g. hepatoblastoma, jaundice, hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells). Protein, as well as, antibodies directed against the protein may show utility as a tissue-specific marker and/or immunotherapy target for the above listed tissues. Moreover, the detected biological activity of the protein product of this gene indicates it is useful for treatment of a variety of immune system disorders. Representative uses are described in the "Immune Activity" and "infectious disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses).

Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such



as AIDS, leukemia, rheumatoid arthritis, disease, sepsis, acne, neutropenia, ne T-cell mediated cytotoxicity; immun such as host-versus-graft and graft-v-

5 such as autoimmune infertility, lense erythematosis, drug induced hemoly Disease, and scleroderma. Moreover influences the differentiation or beha hematopoietic cells to sites of injury.

10 in the expansion of stem cells and co and in the differentiation and/or prol protein may also be used to determin markers, to isolate cognate ligands o interactions, in addition to its use as

15 antibodies directed against the protei immunotherapy targets for the above

Many polynucleotide sequen available and accessible through seq related to SEQ ID NO:36 and may h

20 the present invention. Preferably, suc excluded from the scope of the prese cumbersome. Accordingly, preferabl more polynucleotides comprising a r formula of a-b, where a is any intege

25 integer of 15 to 1815, where both a a residues shown in SEQ ID NO:36, a

## FEATURES OF PROTEIN ENCC

The translation product of thi

30 fatty acid desaturase which is thought into specific positions in fatty acids (

references and information available through this accession are hereby incorporated by reference herein). Preferred polypeptide encoded by this gene comprise the following amino acid sequence:

MGNSASRNDFEWVYTDQPHTQRRARPPAKYPAIKALMRPDPRLKWAVLVL  
5 VLVQMLACWLVRGLAWRWLLFWAYAFGGCVNHSLTLAIHDISHNAAFGTG  
RAARNRWLAVFANLPVGVPIYAASFKKYHVDHHRYLGGDGLDVDVPTRLEG  
WFFCTPARKLLWLVLQPPFYSLRPLCVHPKAVTRMEVLNTLVQLAADLAIFA  
LWGLKPVVYLLASSFLGLGLHPISGHFVAEHYMFLLKGHETYSYGGPLNWITF  
NVGYHVEHHDFFSIPGYNLPLVRKIAPEYYDHLPPQHHSWVKVLWDFVFEDSL  
10 GPYARVKRVYRLAKDGL (SEQ ID NO: 130). Also preferred are polypeptides  
comprising residues 67-197 of the above sequence and polypeptides comprising  
residues 216-323 of the above sequence. Polynucleotides encoding such polypeptides  
are also provided.

In another embodiment, polypeptides comprising the amino acid sequence of  
15 the open reading frame upstream of the predicted signal peptide are contemplated by  
the present invention. Specifically, polypeptides of the invention comprise the  
following amino acid sequences:

LQVPVRNSRVDPRVRAVRAPNGASRPTMGNSASRNDFEWVYTDQPHTQRRR  
RPPAKYPAIKALMRPDPRLKWAVLVLVLVQMLACWLVRGLAWRWLLFWA  
20 YAFGGCVNHSLTLAIHDISHNAAFGTGRAARNRWLAVFANLPVGVPIYAASF  
KKYHVDHHRYLGGDGLDVDVPTRLEGWFFCTPARKLLWLVLQPPFYSLRPL  
CVHPKAVTRMEVLNTLVQLAADLAIFALWGLKPVVYLLASSFLGLGLHPISG  
HFVAEHYMFLLKGHETYSYGGPLNWITFNVGYHVEHHDFFSIPGYNLPLVRKI  
APEYYDHLPPQHHSWVKVLWDFVFEDSLGPYARVKRVYRLAKDGL (SEQ ID  
25 NO: 131); and LQVPVRNSRVDPRVRAVRAPNGASRPT (SEQ ID NO: 132).

Polynucleotides encoding these polypeptides are also provided. Such polypeptides  
find use as further herein described.

The polypeptide of this gene has been determined to have a transmembrane  
domains at about amino acid position 45 - 61 and 198 - 214 of the amino acid  
30 sequence referenced in Table 1 for this gene. Based upon these characteristics, it is  
believed that the protein product of this gene shares structural features of type IIIa

membrane proteins.

This gene is expressed primarily in normal and cancerous tissue from testes, colon, and pancreas.

Therefore, polynucleotides and polypeptides of the invention are useful as  
5 reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, testicular, colon, and pancreatic cancer, as well as male reproductive and endocrine disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential  
10 identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the testes and colon, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an  
15 individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 74 as residues: Met-1 to Arg-6, Arg-65 to Arg-70,  
20 Gly-203 to Tyr-210. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution of this gene in testes and the homology to MLD indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of male reproductive and endocrine disorders. It may also prove to be valuable in the diagnosis and treatment of testicular cancer. It has  
25 been determined that MLD overexpression inhibits the biosynthesis of the EGF receptor, suggesting a possible role of a fatty acid desaturase in regulating biosynthetic processing of the EGF receptor, and by extension the growth and function of epidermal cells. Thus, it is likely that polypeptides and polynucleotides of the present invention share at least some biological activities with the MLD protein.  
30 Such activities are known in the art, some of which are referenced elsewhere herein. Furthermore, the protein may also be used to determine biological activity, to raise

antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement.

Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

5 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:37 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is  
10 cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1452 of SEQ ID NO:37, b is an integer of 15 to 1466, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:37, and where b is greater than or equal to a + 14.

#### 15 **FEATURES OF PROTEIN ENCODED BY GENE NO: 28**

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the  
20 following amino acid sequences:

GFSFSTSLPTLVIFWVFLIIAFLMDMKWFLIVVLICIPMTSDIEHLFMCLLPFH  
VSSLXKCLFKSFAHFSVGLYFVVEF (SEQ ID NO: 133); and

GFSFSTSLPTLVIFWVFLIIAFL (SEQ ID NO: 134). Polynucleotides encoding these polypeptides are also provided.

25 The polypeptide of this gene has been determined to have a transmembrane domain at about amino acid position 5 - 21 of the amino acid sequence referenced in Table 1 for this gene. Moreover, a cytoplasmic tail encompassing amino acids 22 - 57 of this protein has also been determined. Based upon these characteristics, it is believed that the protein product of this gene shares structural features of type Ia  
30 membrane proteins.

This gene is expressed primarily in bone marrow.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, anemias (leukemias) and other hematopoietic and immune disorders and deficiencies. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g. bone, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

The tissue distribution indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of immune disorders including: leukemias, lymphomas, auto-immunities, immunodeficiencies (e.g. AIDS), immuno-suppressive conditions (transplantation) and hematopoietic disorders. In addition this gene product is applicable in conditions of general microbial infection, inflammation or cancer. Representative uses are described in the "Immune Activity" and "infectious disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia.

The gene product may also be involved in lymphopoiesis, therefore, it can be used in immune disorders such as infection, inflammation, allergy, immunodeficiency etc. In addition, this gene product may have commercial utility in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies

directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:38 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1112 of SEQ ID NO:38, b is an integer of 15 to 1126, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:38, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 29

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequences:

RQLPECPPSCAVSCWHWDEDMALVWLCFLNSVEGFGVSRAPPLSPPLEENAQ  
DSGASFRYRKTIALFWTQFSVTSSL (SEQ ID NO:135 and 136); and  
RQLPECPPSCAVSCWHWDED (SEQ ID NO:137). Polynucleotides encoding these polypeptides are also provided.

This gene is expressed primarily in neutrophils and endothelial cells.

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immunodeficiency, infection, lymphomas, auto-immunities, cancer, metastasis, anemias (leukemia) and other hematopoietic disorders. Alternatively, the protein is useful in the detection, treatment, and/or prevention of vascular conditions, which include, but are not limited to, microvascular disease, vascular leak syndrome, aneurysm, stroke, atherosclerosis, arteriosclerosis, or embolism. For example, this

gene product may represent a soluble factor produced by smooth muscle that regulates the innervation of organs or regulates the survival of neighboring neurons. Likewise, it is involved in controlling the digestive process, and such actions as peristalsis. Similarly, it is involved in controlling the vasculature in areas where smooth muscle surrounds the endothelium of blood vessels. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune system, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g. immune, cancerous and wounded tissues) or bodily fluids (e.g. lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 76 as residues: Leu-27 to Gln-32, Phe-38 to Thr-43.

Polynucleotides encoding said polypeptides are also provided.

The tissue distribution indicates that the protein products of this gene are useful for the diagnosis and treatment of immune and vascular disorders including: leukemias, lymphomas, auto-immunities, immunodeficiencies (e.g. AIDS), immunosuppressive conditions (transplantation), hematopoietic disorders, circulatory disorders, metastatic processes. In addition this gene product is applicable in conditions of general microbial infection, inflammation or cancer. Representative uses are described in the "Immune Activity" and "infectious disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. Briefly, the expression of this gene product indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells. This gene product is involved in the regulation of cytokine

production, antigen presentation, or other processes suggesting a usefulness in the treatment of cancer (e.g. by boosting immune responses).

Since the gene is expressed in cells of lymphoid origin, the natural gene product is involved in immune functions. Therefore it is also useful as an agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous Disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosus, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's Disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:39 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2544 of SEQ ID NO:39, b is an integer of 15 to 2558, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:39, and where b is greater than or equal to a + 14.



**FEATURES OF PROTEIN ENCODED BY GENE NO: 30**

This gene is expressed primarily in cells of hematopoietic origin (T-cells, B-cells, macrophages, dendritic cells) and endothelial cells.

5 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immunodeficiency, lymphomas, auto-immunities, cancer, metastasis, anemias (leukemia) and other hematopoietic disorders as well as cardiovascular and  
10 respiratory or pulmonary disorders. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the immune and cardiovascular system, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues  
15 or cell types (e.g. vascular, hematopoietic, immune, and cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

20 Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 77 as residues: Val-30 to Asn-44. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in T-cells, B-cells, macrophages, and dendritic cells indicates that polynucleotides and polypeptides corresponding to this gene are useful  
25 for the diagnosis and treatment of immune disorders including: leukemias, lymphomas, auto-immunities, immunodeficiencies (e.g. AIDS), immuno-suppressive conditions (transplantation) and hematopoietic disorders. Representative uses are described in the "Immune Activity" and "infectious disease" sections below, in Example 11, 13, 14, 16, 18, 19, 20, and 27, and elsewhere herein. In addition this  
30 gene product is applicable in conditions of general microbial infection, inflammation or cancer. The expression in endothelial cells might suggest a role in the treatment

and diagnosis of cardiovascular and respiratory or pulmonary disorders such as athesma, pulmonary edema, pneumonia, atherosclerosis, restenosis, stoke, angina, thrombosis hypertension, inflammation and wound healing. Alternatively, the protein is useful in the detection, treatment, and/or prevention of vascular conditions, which  
5 include, but are not limited to, microvascular disease, vascular leak syndrome, aneurysm, stroke, atherosclerosis, arteriosclerosis, or embolism. For example, this gene product may represent a soluble factor produced by smooth muscle that regulates the innervation of organs or regulates the survival of neighboring neurons. Likewise, it is involved in controlling the digestive process, and such actions as  
10 peristalsis. Similarly, it is involved in controlling the vasculature in areas where smooth muscle surrounds the endothelium of blood vessels. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as,  
15 antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:40 and may have been publicly available prior to conception of  
20 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1925 of SEQ ID NO:40, b is an  
25 integer of 15 to 1939, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:40, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 31

Preferred polypeptides encoded by this gene comprise the following amino  
30 acid sequence:

QGGGGLQAALLALEVGLVGLGASYLLLCTALHLPSSLFLLLAQG TALGAVLG

LSWRRGLMGVPLGLGAAWLLAWPGLALPLVAMAAGGRWVRQQGPRVRRG  
ISRLWLRVLLRLSPMAFRALQGCGAVGDRGLFALYPKTNKDGFRSRLPVPGP  
RRRNPRTTQHPLALLARVWVLCKGWNWRLARASQGLASHLPPWAIHTLAS  
WGLLRGERPPESPGYYHAASAS (SEQ ID NO: 140). Polynucleotides encoding

5 such polypeptides are also provided.

In another embodiment, polypeptides comprising the amino acid sequence of the open reading frame upstream of the predicted signal peptide are contemplated by the present invention. Specifically, polypeptides of the invention comprise the following amino acid sequence:

10 HEVGSSSGLLPLLLLLLLPLLAAQGGGGLQAALLALEVGLVGLGASYLLCT  
ALHLPSSLFLLLAQGTALGAVLGLSWRRGLMGVPLGLGAAWLLAWPGLALP  
LVAMAAGGRWVRQQGPRVRRGISRLWLRVLLRLSPMAFRALQGCGAVGDR  
GLFALYPKTNKDGFRSRLPVPGP  
15 LPGTLAGRRSRTRQSRALPPWR (SEQ ID NO: 138). Polynucleotides encoding these polypeptides are also provided.

A preferred polypeptide variant of the invention comprises the following amino acid sequence:

20 MGVPLGLGAAWLLAWPGLALPLVAMAAGGRWVRQQGPRVRRGISRLWLR  
VLLRLSPMAFRALQGCGAVGDRGLFALYPKTNKDGFRSRLPVPGP  
TQHPLALLARVWVLCKGWNWRLARASQGLASHLPPWAIHTLASWGLLRGE  
RPESPGYYHAASAS (SEQ ID NO: 139). Polynucleotides encoding these polypeptides are also provided.

This gene is expressed primarily in the brain and T-cells.

25 Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include, but are not limited to, immunodeficiency, lymphomas, auto-immunities, cancer, metastasis, inflammation, anemias (leukemia) and other hematopoietic disorders, developmental  
30 and neurodegenerative diseases of the brain and nervous system such as, schizophrenia, Alzheimer's Disease, Parkinson's Disease, Huntington's Disease,

mania, dementia, paranoia, addictive behavior. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the brain and immune system, expression of this gene at significantly higher or lower levels is routinely detected in certain tissues or cell types (e.g. brain, immune, cancerous and wounded tissues) or bodily fluids (e.g., lymph, serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or cell sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Preferred polypeptides of the present invention comprise immunogenic epitopes shown in SEQ ID NO: 78 as residues: Arg-30 to Gly-42, Pro-78 to Arg-88, Pro-92 to Gln-103, Arg-149 to Ile-156, Leu-160 to Leu-167, Ala-171 to Leu-176, Arg-183 to Ala-192. Polynucleotides encoding said polypeptides are also provided.

The tissue distribution in T-cells indicates that polynucleotides and polypeptides corresponding to this gene are useful for the diagnosis and treatment of immune disorders including: leukemias, lymphomas, auto-immunities, immunodeficiencies (e.g. AIDS), immuno-suppressive conditions (transplantation) and hematopoietic disorders. In addition this gene product is applicable in conditions of general microbial infection, inflammation or cancer. The expression in the brain would suggest an application in the treatment and diagnosis of developmental, degenerative and behavioral conditions of the brain and nervous system, such as depression, schizophrenia, Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, transmissible spongiform encephalopathy (TSE), Creutzfeldt-Jakob disease (CJD), Tourette Syndrome, mania, paranoia, addictive behavior, obsessive-compulsive disorder, sleep disorders and dementia.

Moreover, the tissue distribution in brain indicates that the protein product of the gene is useful for the treatment, detection, and/or prevention of neural diseases and/or disorders. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in Example 11, 15, and 18, and elsewhere herein. Briefly, the uses include, but are not limited to the detection,

treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function.

Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:41 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence is cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1215 of SEQ ID NO:41, b is an integer of 15 to 1229, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:41, and where b is greater than or equal to a + 14.

| Gene No. | cDNA Clone ID | ATCC Deposit Nr and Date | Vector        | NT SEQ ID NO: X | Total NT Seq. | 5' NT of Clone Seq. | 3' NT of Clone Seq. | 5' NT of Start Codon | 5' NT of AA of Signal Pep | AA SEQ ID NO: Y | First AA of Sig Pep | Last AA of Sig Pep | First AA of Secreted Portion | Last AA of ORF |
|----------|---------------|--------------------------|---------------|-----------------|---------------|---------------------|---------------------|----------------------|---------------------------|-----------------|---------------------|--------------------|------------------------------|----------------|
| 1        | HCESA34       | 203181<br>09/09/98       | pBluescript   | 11              | 2007          | 1                   | 2002                | 80                   | 80                        | 48              | 1                   | 29                 | 30                           | 40             |
| 2        | HCRMZ90       | 203181<br>09/09/98       | pSportI       | 12              | 1813          | 1                   | 1813                | 161                  | 161                       | 49              | 1                   | 38                 | 39                           | 293            |
| 3        | HDPXQ54       | 203181<br>09/09/98       | pCMVSPORT 3.0 | 13              | 2808          | 1                   | 2808                | 33                   | 33                        | 50              | 1                   | 25                 | 26                           | 118            |
| 4        | HETCL11       | 203181<br>09/09/98       | Uni-ZAP XR    | 14              | 1389          | 1                   | 1389                | 187                  | 187                       | 51              | 1                   | 28                 | 29                           | 76             |
| 5        | HFXDN34       | 203181<br>09/09/98       | Lambda ZAP II | 15              | 1873          | 1                   | 1873                | 185                  | 185                       | 52              | 1                   | 21                 | 22                           | 69             |
| 5        | HFXDN34       | 203364<br>10/19/98       | Lambda ZAP II | 42              | 1897          | 1                   | 1886                | 256                  | 256                       | 79              | 1                   | 21                 | 22                           | 62             |
| 6        | HSDIV86       | 203181<br>09/09/98       | Uni-ZAP XR    | 16              | 2409          | 1                   | 2409                | 204                  | 204                       | 53              | 1                   | 29                 | 30                           | 319            |
| 7        | HMTBE31       | 203181<br>09/09/98       | pCMVSPORT 3.0 | 17              | 1590          | 1                   | 1590                | 25                   | 25                        | 54              | 1                   | 23                 | 24                           | 96             |
| 8        | HRADL70       | 203181<br>09/09/98       | pCMVSPORT 3.0 | 18              | 1567          | 1                   | 1567                | 82                   | 82                        | 55              | 1                   | 18                 | 19                           | 372            |
| 9        | HTXGG31       | 203181<br>09/09/98       | Uni-ZAP XR    | 19              | 3430          | 1                   | 1965                | 38                   | 38                        | 56              | 1                   | 1                  | 2                            | 491            |

| Gene No. | cDNA Clone ID | ATCC Deposit Nr and Date       | Vector        | NT SEQ ID NO: X | Total NT Seq. | 5' NT of Clone Seq. | 3' NT of Clone Seq. | 5' NT of Start Codon | 5' NT of First AA of Signal Pep | AA SEQ ID NO: Y | First AA of Sig Pep | Last AA of Sig Pep | First AA of Secreted Portion | Last AA of ORF |
|----------|---------------|--------------------------------|---------------|-----------------|---------------|---------------------|---------------------|----------------------|---------------------------------|-----------------|---------------------|--------------------|------------------------------|----------------|
| 10       | HWHHL34       | 203181 09/09/98                | pCMVSPORT 3.0 | 20              | 1529          | 95                  | 1529                | 131                  | 131                             | 57              | 1                   | 30                 | 31                           | 188            |
| 10       | HWHHL34       | 203181 09/09/98                | pCMVSPORT 3.0 | 43              | 1796          | 1                   | 1796                | 209                  | 209                             | 80              | 1                   | 31                 | 32                           | 102            |
| 10       | HETGL41       | 97903 02/26/97 209049 05/15/97 | Uni-ZAP XR    | 44              | 2136          | 110                 | 2134                | 296                  | 296                             | 81              | 1                   | 23                 | 24                           | 123            |
| 11       | HYAAY40       | 203181 09/09/98                | pCMVSPORT 3.0 | 21              | 2425          | 1                   | 2425                | 63                   | 63                              | 58              | 1                   | 19                 | 20                           | 40             |
| 12       | HPASA81       | 203181 09/09/98                | Uni-ZAP XR    | 22              | 1971          | 2                   | 1971                | 14                   | 14                              | 59              | 1                   | 17                 | 18                           | 315            |
| 12       | HPASA81       | 203181 09/09/98                | Uni-ZAP XR    | 45              | 2081          | 1                   | 2081                | 124                  | 124                             | 82              | 1                   | 17                 | 18                           | 72             |
| 13       | HCNDA61       | 203181 09/09/98                | Lambda ZAP II | 23              | 1130          | 1                   | 1130                | 63                   | 63                              | 60              | 1                   | 23                 | 24                           | 327            |
| 13       | HCNDA61       | 203181 09/09/98                | Lambda ZAP II | 46              | 1135          | 1                   | 1135                | 50                   | 50                              | 83              | 1                   | 23                 | 24                           | 245            |
| 14       | HTHCZ41       | 203181 09/09/98                | Uni-ZAP XR    | 24              | 1438          | 1                   | 1438                | 14                   | 14                              | 61              | 1                   | 22                 | 23                           | 91             |

| Gene No. | cDNA Clone ID | ATCC Deposit Nr and Date | Vector        | NT SEQ ID NO: X | Total NT Seq. | 5' NT of Clone Seq. | 3' NT of Clone Seq. | 5' NT of Start Codon | 5' NT of First AA of Signal Pep | AA SEQ ID NO: Y | First AA of Sig Pep | Last AA of Sig Pep | First AA of Secreted Portion | Last AA of ORF |
|----------|---------------|--------------------------|---------------|-----------------|---------------|---------------------|---------------------|----------------------|---------------------------------|-----------------|---------------------|--------------------|------------------------------|----------------|
| 15       | HKADJ17       | 203181<br>09/09/98       | pCMVSPORT 2.0 | 25              | 916           | 1                   | 916                 | 67                   | 67                              | 62              | 1                   | 17                 | 18                           | 58             |
| 16       | HMSII78       | 203181<br>09/09/98       | Uni-ZAP XR    | 26              | 2094          | 178                 | 2055                | 315                  | 315                             | 63              | 1                   | 23                 | 24                           | 511            |
| 17       | HCFBL76       | 203181<br>09/09/98       | pSport1       | 27              | 2076          | 1                   | 2076                | 269                  | 269                             | 64              | 1                   | 20                 | 21                           | 90             |
| 18       | HFVHR84       | 203181<br>09/09/98       | pBluescript   | 28              | 1378          | 1                   | 1378                | 155                  | 155                             | 65              | 1                   | 16                 | 17                           | 113            |
| 19       | HIBCB67       | 203181<br>09/09/98       | Other         | 29              | 1965          | 1                   | 1965                | 313                  | 313                             | 66              | 1                   | 29                 | 30                           | 50             |
| 20       | HLHCC76       | 203181<br>09/09/98       | Uni-ZAP XR    | 30              | 1473          | 1                   | 1472                | 143                  | 143                             | 67              | 1                   | 30                 | 31                           | 215            |
| 21       | HAHDZ77       | 203181<br>09/09/98       | Uni-ZAP XR    | 31              | 1157          | 1                   | 1157                | 104                  | 104                             | 68              | 1                   | 18                 | 19                           | 311            |
| 22       | HDHMA45       | 203331<br>10/08/98       | pCMVSPORT 2.0 | 32              | 2190          | 1                   | 2190                | 204                  | 204                             | 69              | 1                   | 33                 | 34                           | 413            |
| 23       | HELA W45      | 203331<br>10/08/98       | Uni-ZAP XR    | 33              | 2511          | 1                   | 2511                | 38                   | 38                              | 70              | 1                   | 21                 | 22                           | 60             |
| 24       | HFIAB31       | 203331<br>10/08/98       | pSport1       | 34              | 1684          | 1                   | 1684                | 36                   | 36                              | 71              | 1                   | 22                 | 23                           | 68             |



| Gene No. | cDNA Clone ID | ATCC Deposit Nr and Date | Vector        | NT SEQ ID NO: X | Total NT Seq. | 5' NT of Clone Seq. | 3' NT of Clone Seq. | 5' NT of Start Codon | 5' NT of First AA of Signal Pep | AA SEQ ID NO: Y | First AA of Sig Pep | Last AA of Sig Pep | First AA of Secreted Portion | Last AA of ORF |
|----------|---------------|--------------------------|---------------|-----------------|---------------|---------------------|---------------------|----------------------|---------------------------------|-----------------|---------------------|--------------------|------------------------------|----------------|
| 25       | HLWBK05       | 203331<br>10/08/98       | pCMVSPORT 3.0 | 35              | 2383          | 157                 | 2383                | 280                  | 280                             | 72              | 1                   | 34                 | 35                           | 298            |
| 26       | HLDBX13       | 203331<br>10/08/98       | pCMVSPORT 3.0 | 36              | 1815          | 1                   | 1815                | 303                  | 303                             | 73              | 1                   | 39                 | 40                           | 55             |
| 27       | HMAGA15       | 203331<br>10/08/98       | Uni-ZAP XR    | 37              | 1466          | 153                 | 1441                | 192                  | 192                             | 74              | 1                   | 39                 | 40                           | 287            |
| 28       | HMWFT53       | 203331<br>10/08/98       | Uni-ZAP XR    | 38              | 1126          | 1                   | 1126                | 97                   | 97                              | 75              | 1                   | 19                 | 20                           | 57             |
| 29       | HNFJD91       | 203331<br>10/08/98       | Uni-ZAP XR    | 39              | 2558          | 1                   | 2558                | 210                  | 210                             | 76              | 1                   | 14                 | 15                           | 58             |
| 30       | HTGCM55       | 203331<br>10/08/98       | Uni-ZAP XR    | 40              | 1939          | 1                   | 1939                | 22                   | 22                              | 77              | 1                   | 32                 | 33                           | 50             |
| 31       | HTTEX77       | 203331<br>10/08/98       | Uni-ZAP XR    | 41              | 1229          | 1                   | 1229                | 249                  | 249                             | 78              | 1                   | 26                 | 27                           | 197            |
| 31       | HTTEX77       | 203331<br>10/08/98       | Uni-ZAP XR    | 47              | 1227          | 1                   | 1227                | 249                  | 249                             | 84              | 1                   | 21                 | 22                           | 166            |

Table 1 summarizes the information corresponding to each "Gene No." described above. The nucleotide sequence identified as "NT SEQ ID NO:X" was assembled from partially homologous ("overlapping") sequences obtained from the "cDNA clone ID" identified in Table 1 and, in some cases, from additional related DNA clones. The overlapping sequences were assembled into a single contiguous sequence of high redundancy (usually three to five overlapping sequences at each nucleotide position), resulting in a final sequence identified as SEQ ID NO:X.

The cDNA Clone ID was deposited on the date and given the corresponding deposit number listed in "ATCC Deposit No:Z and Date." Some of the deposits contain multiple different clones corresponding to the same gene. "Vector" refers to the type of vector contained in the cDNA Clone ID.

"Total NT Seq." refers to the total number of nucleotides in the contig identified by "Gene No." The deposited clone may contain all or most of these sequences, reflected by the nucleotide position indicated as "5' NT of Clone Seq." and the "3' NT of Clone Seq." of SEQ ID NO:X. The nucleotide position of SEQ ID NO:X of the putative start codon (methionine) is identified as "5' NT of Start Codon." Similarly, the nucleotide position of SEQ ID NO:X of the predicted signal sequence is identified as "5' NT of First AA of Signal Pep."

The translated amino acid sequence, beginning with the methionine, is identified as "AA SEQ ID NO:Y," although other reading frames can also be easily translated using known molecular biology techniques. The polypeptides produced by these alternative open reading frames are specifically contemplated by the present invention.

The first and last amino acid position of SEQ ID NO:Y of the predicted signal peptide is identified as "First AA of Sig Pep" and "Last AA of Sig Pep." The predicted first amino acid position of SEQ ID NO:Y of the secreted portion is identified as "Predicted First AA of Secreted Portion." Finally, the amino acid position of SEQ ID NO:Y of the last amino acid in the open reading frame is identified as "Last AA of ORF."

SEQ ID NO:X (where X may be any of the polynucleotide sequences disclosed in the sequence listing) and the translated SEQ ID NO:Y (where Y may be

any of the polypeptide sequences disclosed in the sequence listing) are sufficiently accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, SEQ ID NO:X is useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:X or the cDNA contained in the deposited clone. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:Y may be used, for example, to generate antibodies which bind specifically to proteins containing the polypeptides and the secreted proteins encoded by the cDNA clones identified in Table 1.

Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:X and the predicted translated amino acid sequence identified as SEQ ID NO:Y, but also a sample of plasmid DNA containing a human cDNA of the invention deposited with the ATCC, as set forth in Table 1. The nucleotide sequence of each deposited clone can readily be determined by sequencing the deposited clone in accordance with known methods. The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by a particular clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human cDNA, collecting the protein, and determining its sequence.

The present invention also relates to the genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or the deposited clone. The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include preparing probes or primers from the disclosed  
5 sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

Also provided in the present invention are allelic variants, orthologs, and/or species homologs. Procedures known in the art can be used to obtain full-length genes, allelic variants, splice variants, full-length coding portions, orthologs, and/or  
10 species homologs of genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or a deposited clone, using information from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or  
15 the desired homologue.

The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides  
20 are well understood in the art.

The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification ,  
25 such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially  
30 purified using techniques described herein or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson, Gene 67:31-40

(1988). Polypeptides of the invention also can be purified from natural, synthetic or recombinant sources using techniques described herein or otherwise known in the art, such as, for example, antibodies of the invention raised against the secreted protein.

The present invention provides a polynucleotide comprising, or alternatively  
5 consisting of, the nucleic acid sequence of SEQ ID NO:X, and/or a cDNA contained in ATCC deposit Z. The present invention also provides a polypeptide comprising, or alternatively, consisting of, the polypeptide sequence of SEQ ID NO:Y and/or a polypeptide encoded by the cDNA contained in ATCC deposit Z. Polynucleotides encoding a polypeptide comprising, or alternatively consisting of the polypeptide  
10 sequence of SEQ ID NO:Y and/or a polypeptide sequence encoded by the cDNA contained in ATCC deposit Z are also encompassed by the invention.

#### Signal Sequences

The present invention also encompasses mature forms of the polypeptide having the polypeptide sequence of SEQ ID NO:Y and/or the polypeptide sequence  
15 encoded by the cDNA in a deposited clone. Polynucleotides encoding the mature forms (such as, for example, the polynucleotide sequence in SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone) are also encompassed by the invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretary leader sequence which is cleaved from  
20 the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species of the protein. Further, it has long been known that cleavage  
25 specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide.

Methods for predicting whether a protein has a signal sequence, as well as the cleavage point for that sequence, are available. For instance, the method of  
30 McGeoch, Virus Res. 3:271-286 (1985), uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved)

protein. The method of von Heinje, *Nucleic Acids Res.* 14:4683-4690 (1986) uses the information from the residues surrounding the cleavage site, typically residues -13 to +2, where +1 indicates the amino terminus of the secreted protein. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. (von Heinje, *supra.*) However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

In the present case, the deduced amino acid sequence of the secreted polypeptide was analyzed by a computer program called SignalP (Henrik Nielsen et al., *Protein Engineering* 10:1-6 (1997)), which predicts the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis of the amino acid sequences of the secreted proteins described herein by this program provided the results shown in Table 1.

As one of ordinary skill would appreciate, however, cleavage sites sometimes vary from organism to organism and cannot be predicted with absolute certainty. Accordingly, the present invention provides secreted polypeptides having a sequence shown in SEQ ID NO:Y which have an N-terminus beginning within 5 residues (i.e., + or - 5 residues) of the predicted cleavage point. Similarly, it is also recognized that in some cases, cleavage of the signal sequence from a secreted protein is not entirely uniform, resulting in more than one secreted species. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Moreover, the signal sequence identified by the above analysis may not necessarily predict the naturally occurring signal sequence. For example, the naturally occurring signal sequence may be further upstream from the predicted signal sequence. However, it is likely that the predicted signal sequence will be capable of directing the secreted protein to the ER. Nonetheless, the present invention provides the mature protein produced by expression of the polynucleotide sequence of SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone, in a mammalian cell (e.g., COS cells, as described below). These polypeptides,

and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

### **Polynucleotide and Polypeptide Variants**

5       The present invention is directed to variants of the polynucleotide sequence disclosed in SEQ ID NO:X, the complementary strand thereto, and/or the cDNA sequence contained in a deposited clone.

      The present invention also encompasses variants of the polypeptide sequence disclosed in SEQ ID NO:Y and/or encoded by a deposited clone.

10       "Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

      The present invention is also directed to nucleic acid molecules which  
15       comprise, or alternatively consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for example, the nucleotide coding sequence in SEQ ID NO:X or the complementary strand thereto, the nucleotide coding sequence contained in a deposited cDNA clone or the  
20       complementary strand thereto, a nucleotide sequence encoding the polypeptide of SEQ ID NO:Y, a nucleotide sequence encoding the polypeptide encoded by the cDNA contained in a deposited clone, and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., those fragments described herein).

      Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the  
25       invention, as are polypeptides encoded by these polynucleotides.

      The present invention is also directed to polypeptides which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, for example, the polypeptide sequence shown in SEQ ID NO:Y, the polypeptide sequence encoded by the cDNA contained  
30       in a deposited clone, and/or polypeptide fragments of any of these polypeptides (e.g., those fragments described herein).

By a nucleic acid having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the nucleic acid is identical to the reference sequence except that the nucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the polypeptide. In other words, to obtain a nucleic acid having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence shown in Table 1, the ORF (open reading frame), or any fragment specified as described herein.

As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245(1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject



sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is  
5 determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment,  
10 which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a  
15 matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base  
20 subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence  
25 are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence  
30 except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other

words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy

5 terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, an amino acid  
10 sequences shown in Table 1 (SEQ ID NO:Y) or to the amino acid sequence encoded by cDNA contained in a deposited clone can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using  
15 the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245(1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch  
20 Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be  
25 made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are  
30 not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by

results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred.

Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced

for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. The authors of Ron et al., *J. Biol. Chem.* 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., *J. Biotechnology* 7:199-216 (1988).)

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (*J. Biol. Chem* 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Thus, the invention further includes polypeptide variants which show substantial biological activity. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as to have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of the present invention having an amino acid sequence which contains at least one amino acid substitution, but not more than 50

amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a peptide or polypeptide  
5 to have an amino acid sequence which comprises the amino acid sequence of the present invention, which contains at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitutions. In specific embodiments, the number of additions, substitutions, and/or deletions in the amino acid sequence of the present invention or fragments thereof (e.g., the mature form and/or other fragments described herein), is  
10 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative amino acid substitutions are preferable.

#### **Polynucleotide and Polypeptide Fragments**

The present invention is also directed to polynucleotide fragments of the  
15 polynucleotides of the invention.

In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence which: is a portion of that contained in a deposited clone, or encoding the polypeptide encoded by the cDNA in a deposited clone; is a portion of that shown in SEQ ID NO:X or the complementary strand  
20 thereto, or is a portion of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:Y. The nucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, or at least about 150 nt in length. A fragment "at least 20 nt in length," for  
25 example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in a deposited clone or the nucleotide sequence shown in SEQ ID NO:X. In this context "about" includes the particularly recited value, a value larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. These nucleotide fragments have uses that include, but are not limited to, as  
30 diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, 500, 600, 2000 nucleotides) are preferred.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, 701-750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, or 2001 to the end of SEQ ID NO:X, or the complementary strand thereto, or the cDNA contained in a deposited clone. In this context "about" includes the particularly recited ranges, and ranges larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini.

Preferably, these fragments encode a polypeptide which has biological activity. More preferably, these polynucleotides can be used as probes or primers as discussed herein. Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of that contained in SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, or 161 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, and ranges or values larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Preferred polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or



the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form.

Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also preferred.

Also preferred are polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions.

Polypeptide fragments of SEQ ID NO:Y falling within conserved domains are specifically contemplated by the present invention. Moreover, polynucleotides encoding these domains are also contemplated.

Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

Preferably, the polynucleotide fragments of the invention encode a polypeptide which demonstrates a functional activity. By a polypeptide demonstrating a "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) polypeptide of invention protein. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide of the invention for binding) to an antibody to the polypeptide of the invention], immunogenicity (ability to generate antibody which binds to a polypeptide

of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide of the invention.

The functional activity of polypeptides of the invention, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

5 For example, in one embodiment where one is assaying for the ability to bind or compete with full-length polypeptide of the invention for binding to an antibody of the polypeptide of the invention, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent  
10 assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays,  
15 protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an  
20 immunoassay and are within the scope of the present invention.

In another embodiment, where a ligand for a polypeptide of the invention identified, or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel  
25 chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky, E., et al., 1995, Microbiol. Rev. 59:94-123. In another embodiment, physiological correlates of binding of a polypeptide of the invention to its substrates (signal transduction) can be assayed.

In addition, assays described herein (see Examples) and otherwise known in  
30 the art may routinely be applied to measure the ability of polypeptides of the invention and fragments, variants derivatives and analogs thereof to elicit related

biological activity related to that of the polypeptide of the invention (either in vitro or in vivo). Other methods will be known to the skilled artisan and are within the scope of the invention.

## 5 Epitopes & Antibodies

The present invention is also directed to polypeptide fragments comprising, or alternatively consisting of, an epitope of the polypeptide sequence shown in SEQ ID NO:Y, or the polypeptide sequence encoded by the cDNA contained in a deposited clone. Polynucleotides encoding these epitopes (such as, for example, the sequence  
10 disclosed in SEQ ID NO:X) are also encompassed by the invention, as is the nucleotide sequences of the complementary strand of the polynucleotides encoding these epitopes. And polynucleotides which hybridize to the complementary strand under stringent hybridization conditions or lower stringency conditions.

In the present invention, "epitopes" refer to polypeptide fragments having  
15 antigenic or immunogenic activity in an animal, especially in a human. A preferred embodiment of the present invention relates to a polypeptide fragment comprising an epitope, as well as the polynucleotide encoding this fragment. A region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." In contrast, an "immunogenic epitope" is defined as a part of a protein that elicits an  
20 antibody response. (See, for instance, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998-4002 (1983).)

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, R. A., Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985) further described in U.S. Patent No. 4,631,211.)

25 In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, and most preferably between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100  
30 amino acid residues in length. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. (See,

for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe et al., Science 219:660-666 (1983).

Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle et al., J. Gen. Virol. 66:2347-2354 (1985).) A preferred immunogenic epitope includes the secreted protein. The immunogenic epitopes may be presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) or, if it is long enough (at least about 25 amino acids), without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting.)

Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, *in vivo* immunization, *in vitro* immunization, and phage display methods. See, e.g., Sutcliffe et al., supra; Wilson et al., supra, and Bittle et al., J. Gen. Virol., 66:2347-2354 (1985). If *in vivo* immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling of the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as -maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier-coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µgs of peptide or carrier protein and Freund's adjuvant. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected

antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and discussed above, the polypeptides of the present invention comprising an immunogenic or antigenic epitope can be fused to heterologous polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, any combination thereof including both entire domains and portions thereof) resulting in chimeric polypeptides. These fusion proteins facilitate purification, and show an increased half-life *in vivo*. This has been shown, *e.g.*, for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, *e.g.*, EPA 0,394,827; Traunecker et al., *Nature*, 331:84-86 (1988). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion can also be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, *e.g.*, Fountoulakis et al., *J. Biochem.*, 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag to aid in detection and purification of the expressed polypeptide.

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides corresponding to SEQ ID NO:Y thereby effectively generating agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten, P.A., et al., *Curr. Opinion Biotechnol.* 8:724-33 (1997); Harayama, S., *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, L.O., et al., *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R., *Biotechniques* 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:X and corresponding polypeptides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired molecule

corresponding to SEQ ID NO:X polynucleotides of the invention by homologous, or site-specific, recombination. In another embodiment, polynucleotides corresponding to SEQ ID NO:X and corresponding polypeptides may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of coding polynucleotide corresponding to SEQ ID NO:X, or the polypeptide encoded thereby may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

### Antibodies

The present invention further relates to antibodies and T-cell antigen receptors (TCR) which specifically bind the polypeptides of the present invention. The antibodies of the present invention include IgG (including IgG1, IgG2, IgG3, and IgG4), IgA (including IgA1 and IgA2), IgD, IgE, or IgM, and IgY. As used herein, the term "antibody" (Ab) is meant to include whole antibodies, including single-chain whole antibodies, and antigen-binding fragments thereof. Most preferably the antibodies are human antigen binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')<sub>2</sub>, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a V<sub>L</sub> or V<sub>H</sub> domain. The antibodies may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine, rabbit, goat, guinea pig, camel, horse, or chicken.

Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entire or partial of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are any combinations of variable region(s) and hinge region, CH1, CH2, and CH3 domains. The present invention further includes monoclonal, polyclonal, chimeric, humanized, and human monoclonal and human polyclonal antibodies which specifically bind the polypeptides of the present invention. The present invention further includes antibodies which are anti-idiotypic to the antibodies of the present

invention.

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both  
5 a polypeptide of the present invention as well as for heterologous compositions, such as a heterologous polypeptide or solid support material. *See, e.g.*, WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); US Patents 5,573,920, 4,474,893, 5,601,819, 4,714,681, 4,925,648; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

10 Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which are recognized or specifically bound by the antibody. The epitope(s) or polypeptide portion(s) may be specified as described herein, *e.g.*, by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and  
15 Figures. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in  
20 terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of the polypeptides of the present invention are included. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described  
25 herein) to a polypeptide of the present invention are also included in the present invention. Further included in the present invention are antibodies which only bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of  
30 their binding affinity. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-6}M$ ,  $10^{-6}M$ ,  $5 \times 10^{-7}M$ ,  $10^{-7}M$ ,  $5 \times 10^{-8}M$ ,  $10^{-8}M$ ,  $5 \times 10^{-9}M$ .

$^9\text{M}$ ,  $10^{-9}\text{M}$ ,  $5 \times 10^{-10}\text{M}$ ,  $10^{-10}\text{M}$ ,  $5 \times 10^{-11}\text{M}$ ,  $10^{-11}\text{M}$ ,  $5 \times 10^{-12}\text{M}$ ,  $10^{-12}\text{M}$ ,  $5 \times 10^{-13}\text{M}$ ,  $10^{-13}\text{M}$ ,  $5 \times 10^{-14}\text{M}$ ,  $10^{-14}\text{M}$ ,  $5 \times 10^{-15}\text{M}$ , and  $10^{-15}\text{M}$ .

Antibodies of the present invention have uses that include, but are not limited to, methods known in the art to purify, detect, and target the polypeptides of the present invention including both *in vitro* and *in vivo* diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., ANTIBODIES: A LABORATORY MANUAL, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference in the entirety).

The antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, or toxins. See, e.g., WO 92/08495; WO 91/14438; WO 89/12624; US Patent 5,314,995; and EP 0 396 387.

The antibodies of the present invention may be prepared by any suitable method known in the art. For example, a polypeptide of the present invention or an antigenic fragment thereof can be administered to an animal in order to induce the production of sera containing polyclonal antibodies. The term "monoclonal antibody" is not a limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technology.

Hybridoma techniques include those known in the art and taught in Harlow et al., ANTIBODIES: A LABORATORY MANUAL, (Cold Spring Harbor Laboratory



Press, 2nd ed. 1988); Hammerling, et al., in: MONOCLONAL ANTIBODIES AND T-CELL HYBRIDOMAS 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). Fab and F(ab')<sub>2</sub> fragments may be produced by proteolytic cleavage, using enzymes such as papain (to produce Fab  
5 fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments).

Alternatively, antibodies of the present invention can be produced through the application of recombinant DNA and phage display technology or through synthetic chemistry using methods known in the art. For example, the antibodies of the present invention can be prepared using various phage display methods known in the art. In  
10 phage display methods, functional antibody domains are displayed on the surface of a phage particle which carries polynucleotide sequences encoding them. Phage with a desired binding property are selected from a repertoire or combinatorial antibody library (e.g. human or murine) by selecting directly with antigen, typically antigen bound or captured to a solid surface or bead. Phage used in these methods are  
15 typically filamentous phage including fd and M13 with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995);  
20 Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT/GB91/01134; WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and US Patents 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908, 5,750,753, 5,821,047, 5,571,698, 5,427,908,  
25 5,516,637, 5,780,225, 5,658,727 and 5,733,743 (said references incorporated by reference in their entireties).

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and  
30 expressed in any desired host including mammalian cells, insect cells, plant cells, yeast, and bacteria. For example, techniques to recombinantly produce Fab, Fab' and

F(ab')<sub>2</sub> fragments can also be employed using methods known in the art such as those disclosed in WO 92/22324; Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-34 (1995); and Better et al., Science 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

5        Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., Methods in Enzymology 203:46-88 (1991); Shu, L. et al., PNAS 90:7995-7999 (1993); and Skerra et al., Science 240:1038-1040 (1988). For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it may be preferable  
10    to use chimeric, humanized, or human antibodies. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol. Methods 125:191-202; and US Patent 5,807,715. Antibodies can be humanized using a variety of techniques including CDR-grafting (EP 0 239 400; WO 91/09967; US Patent  
15    5,530,101; and 5,585,089), veneering or resurfacing (EP 0 592 106; EP 0 519 596; Padlan E.A., Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (US Patent 5,565,332). Human antibodies can be made by a variety of methods known in the art including phage display methods described  
20    above. See also, US Patents 4,444,887, 4,716,111, 5,545,806, and 5,814,318; and WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741 (said references incorporated by reference in their entireties).

25        Further included in the present invention are antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide of the present invention. The antibodies may be specific for antigens other than polypeptides of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either *in vitro* or *in vivo*, by fusing or conjugating the polypeptides of the present invention  
30    to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in *in vitro*

immunoassays and purification methods using methods known in the art. See e.g., Harbor et al. *supra* and WO 93/21232; EP 0 439 095; Naramura et al., Immunol. Lett. 39:91-99 (1994); US Patent 5,474,981; Gillies et al., PNAS 89:1428-1432 (1992); Fell et al., J. Immunol. 146:2446-2452 (1991) (said references incorporated by  
5 reference in their entirety).

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody  
10 portion fused to a polypeptide of the present invention may comprise the hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides of the present invention may be fused or conjugated to the above antibody portions to increase the *in vivo* half life of the polypeptides or for use in immunoassays using methods known in the art. The  
15 polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to  
20 antibody portions are known in the art. See e.g., US Patents 5,336,603, 5,622,929, 5,359,046, 5,349,053, 5,447,851, 5,112,946; EP 0 307 434, EP 0 367 166; WO 96/04388, WO 91/06570; Ashkenazi et al., PNAS 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., PNAS 89:11337-11341 (1992) (said references incorporated by reference in their entirety).

25 The invention further relates to antibodies which act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Included are both receptor-specific antibodies and ligand-specific antibodies. Included are receptor-specific  
30 antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described

herein or otherwise known in the art. Also included are receptor-specific antibodies which both prevent ligand binding and receptor activation. Likewise, included are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included are antibodies which activate the receptor. These antibodies may act as agonists for either all or less than all of the biological activities affected by ligand-mediated receptor activation. The antibodies may be specified as agonists or antagonists for biological activities comprising specific activities disclosed herein. The above antibody agonists can be made using methods known in the art. See e.g., WO 96/40281; US Patent 5,811,097; Deng et al., *Blood* 92(6):1981-1988 (1998); Chen, et al., *Cancer Res.* 58(16):3668-3678 (1998); Harrop et al., *J. Immunol.* 161(4):1786-1794 (1998); Zhu et al., *Cancer Res.* 58(15):3209-3214 (1998); Yoon, et al., *J. Immunol.* 160(7):3170-3179 (1998); Prat et al., *J. Cell. Sci.* 111(Pt2):237-247 (1998); Pitard et al., *J. Immunol. Methods* 205(2):177-190 (1997); Liautard et al., *Cytokine* 9(4):233-241 (1997); Carlson et al., *J. Biol. Chem.* 272(17):11295-11301 (1997); Taryman et al., *Neuron* 14(4):755-762 (1995); Muller et al., *Structure* 6(9):1153-1167 (1998); Bartunek et al., *Cytokine* 8(1):14-20 (1996) (said references incorporated by reference in their entireties).

As discussed above, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, *FASEB J.* 7(5):437-444; (1989) and Nissinoff, *J. Immunol.* 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide

of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

The invention further relates to a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a substantially isolated polypeptide antigen  
5 comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Such a kit also includes means for detecting the binding of said antibody to the antigen. In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The  
10 polypeptide antigen of the kit may also be attached to a solid support.

In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labelled anti-human antibody. In this  
15 embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labelled antibody.

The invention further includes a method of detecting proliferative and/or cancerous disorders and conditions in a test subject. This detection method includes reacting serum from a test subject (e.g. one in which proliferative and/or cancerous cells or tissues may be present) with a substantially isolated polypeptide and/or  
20 polynucleotide antigen, and examining the antigen for the presence of bound antibody. In a specific embodiment, the method includes a polypeptide antigen attached to a solid support, and the serum is reacted with the support. Subsequently, the support is reacted with a reporter-labelled anti-human antibody. The solid support is then examined for the presence of reporter-labelled antibody.

25 Additionally, the invention includes a proliferative condition vaccine composition. The composition includes a substantially isolated polypeptide and/or polynucleotide antigen, where the antigen includes an epitope which is specifically immunoreactive with at least antibody specific for the epitope. The peptide and/or polynucleotide antigen may be produced according to methods known in the art,  
30 including recombinant expression or chemical synthesis. The peptide antigen is

preferably present in a pharmacologically effective dose in a pharmaceutically acceptable carrier.

Further, the invention includes a monoclonal antibody that is specifically immunoreactive with polypeptide and/or polynucleotide epitopes. The invention  
5 includes a substantially isolated preparation of polyclonal antibodies specifically immunoreactive with polynucleotides and/or polypeptides of the present invention. In a more specific embodiment, such polyclonal antibodies are prepared by affinity chromatography, in addition to, other methods known in the art.

In another embodiment, the invention includes a method for producing  
10 antibodies to polypeptide and/or polynucleotide antigens. The method includes administering to a test subject a substantially isolated polypeptide and/or polynucleotide antigen, where the antigen includes an epitope which is specifically immunoreactive with at least one anti- polypeptide and/or polynucleotide antibody. The antigen is administered in an amount sufficient to produce an immune response in  
15 the subject.

In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of  
20 the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labelled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labelled, competing antigen.

25 In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labelled anti-human antibody to bind reporter to the reagent in proportion to the amount of  
30 bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labelled antibody, and the amount of reporter associated with the

reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric or colorimetric substrate (Sigma, St. Louis, MO).

The solid surface reagent in the above assay is prepared by known techniques  
5 for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group.  
10 Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labelled anti-human antibody for detecting  
15 surface-bound anti-antigen antibody.

### **Fusion Proteins**

Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a  
20 second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target cellular locations based on trafficking signals, the polypeptides of the present invention can be used as targeting molecules once fused to other proteins.

25 Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics  
30 of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the

polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling  
5 of polypeptides are familiar and routine techniques in the art.

Moreover, polypeptides of the present invention, including fragments, and specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgA, IgE, IgG, IgM) or portions thereof (CH1, CH2, CH3, and any combination thereof, including both entire domains and portions thereof), resulting in  
10 chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988).)  
15 Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995).)

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion  
20 proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified,  
25 would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol.  
30 Chem. 270:9459-9471 (1995).)



Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984).)

Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

#### **Vectors, Host Cells, and Protein Production**

The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding

portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one  
5 selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells;  
10 insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A,  
15 pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

20 Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., *Basic Methods In Molecular Biology* (1986). It is specifically contemplated that the  
25 polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography,  
30 phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most

preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Polypeptides of the present invention, and preferably the secreted form, can also be recovered from: products purified from natural sources, including bodily  
5 fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated  
10 or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal  
15 methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and  
20 immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with the polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous polynucleotides. For example,  
25 techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al.,  
30 Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-

438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, *Proteins: Structures and*  
5 *Molecular Principles*, W.H. Freeman & Co., N.Y., and Hunkapiller et al., *Nature*, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide sequence of the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence.

10 Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid,  $\alpha$ -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid,  $\gamma$ -Abu,  $\epsilon$ -Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-  
15 butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine,  $\beta$ -alanine, fluoro-amino acids, designer amino acids such as  $\beta$ -methyl amino acids,  $\text{Ca}$ -methyl amino acids,  $\text{Na}$ -methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

The invention encompasses polypeptides which are differentially modified  
20 during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin,  
25 papain, V8 protease,  $\text{NaBH}_4$ ; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid  
30 backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic

host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent NO: 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., Exp. Hematol. 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues;

those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules.

Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

The polypeptides of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions (preferably, *Therapeutics*) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides corresponding to the amino acid sequence of SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone (including fragments, variants, splice variants, and

fusion proteins, corresponding to these polypeptides as described herein). These homomers may contain polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptides having an identical amino acid sequence. In another  
5 specific embodiment, a homomer of the invention is a multimer containing polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (*e.g.*, containing polypeptides having identical or different amino acid sequences) or a homotrimer (*e.g.*, containing polypeptides having identical and/or different amino acid sequences). In additional  
10 embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (*i.e.*, polypeptides of different proteins) in addition to the polypeptides of the invention. In a specific embodiment, the multimer of the  
15 invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example,  
20 liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the  
25 invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence ( *e.g.*, that recited in the sequence  
30 listing, or contained in the polypeptide encoded by a deposited clone). In one instance, the covalent associations are cross-linking between cysteine residues located

within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the  
5 heterologous polypeptide sequence in a fusion protein of the invention.

In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in an Fc fusion protein of the invention (as  
10 described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is capable of forming covalently associated multimers, such as for example, osteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another  
15 embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

20 Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al.,  
25 Science 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant  
30 fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells,



and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention containing Flag® polypeptide sequence. In a further embodiment, associations proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti-Flag® antibody.

The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the C terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see,

e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained  
5 in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the  
10 invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described  
15 herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hydrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

### Uses of the Polynucleotides

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

25 The polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each polynucleotide of the present invention can be used as a chromosome marker.

30 Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NO:X. Primers can be

selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the SEQ ID NO:X will yield an  
5 amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping  
10 strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, and preselection by hybridization to construct chromosome specific-cDNA libraries.

Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread.  
15 This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to  
20 mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes). Preferred polynucleotides correspond to the noncoding regions of the cDNAs because the coding sequences are more likely conserved within gene families, thus increasing the chance of cross hybridization during chromosomal mapping.

25 Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins  
30 University Welch Medical Library) .) Assuming 1 megabase mapping resolution and

one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in the polynucleotide and the corresponding gene between affected and unaffected individuals can be examined.

5 First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide  
10 and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using  
15 polynucleotides of the present invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

Thus, the invention also provides a diagnostic method useful during diagnosis of a disorder, involving measuring the expression level of polynucleotides of the  
20 present invention in cells or body fluid from an individual and comparing the measured gene expression level with a standard level of polynucleotide expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a disorder.

In still another embodiment, the invention includes a kit for analyzing samples  
25 for the presence of proliferative and/or cancerous polynucleotides derived from a test subject. In a general embodiment, the kit includes at least one polynucleotide probe containing a nucleotide sequence that will specifically hybridize with a polynucleotide of the present invention and a suitable container. In a specific embodiment, the kit includes two polynucleotide probes defining an internal region of  
30 the polynucleotide of the present invention, where each probe has one strand

containing a 31' mer-end internal to the region. In a further embodiment, the probes may be useful as primers for polymerase chain reaction amplification.

Where a diagnosis of a disorder, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed polynucleotide of the present invention expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "measuring the expression level of polynucleotide of the present invention" is intended qualitatively or quantitatively measuring or estimating the level of the polypeptide of the present invention or the level of the mRNA encoding the polypeptide in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having a disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains the polypeptide of the present invention or mRNA. As indicated, biological samples include body fluids (such as semen, lymph, sera, plasma, urine, synovial fluid and spinal fluid) which contain the polypeptide of the present invention, and other tissue sources found to express the polypeptide of the present invention. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The method(s) provided above may preferably be applied in a diagnostic method and/or kits in which polynucleotides and/or polypeptides are attached to a

solid support. In one exemplary method, the support may be a "gene chip" or a "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with polynucleotides of the present invention attached may be used to identify polymorphisms between the polynucleotide sequences, with  
5 polynucleotides isolated from a test subject. The knowledge of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying disease loci for many disorders, including cancerous diseases and conditions. Such a method is described in US Patents 5,858,659 and 5,856,104. The US Patents referenced supra are hereby incorporated by reference in their entirety herein.

10 The present invention encompasses polynucleotides of the present invention that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the preferred form if the polynucleotides are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a  
15 polyamide type of DNA analog and the monomeric units for adenine, guanine, thymine and cytosine are available commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, Science 254, 1497 (1991); and M. Egholm, O. Buchardt,  
20 L.Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, Nature 365, 666 (1993), PNAs bind specifically and tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide  
25 backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the strong binding. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer  
30 lowers the melting point (T.sub.m) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can

be done at low ionic strengths and reduce possible interference by salt during the analysis.

The present invention is useful for detecting cancer in mammals. In particular the invention is useful during diagnosis of pathological cell proliferative neoplasias which include, but are not limited to: acute myelogenous leukemias including acute  
5 monocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute erythroleukemia, acute megakaryocytic leukemia, and acute undifferentiated leukemia, etc.; and chronic myelogenous leukemias including chronic myelomonocytic leukemia, chronic granulocytic  
10 leukemia, etc. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Pathological cell proliferative disorders are often associated with inappropriate activation of proto-oncogenes. (Germann, E. P. et al., "The Etiology of Acute Leukemia: Molecular Genetics and Viral Oncology," in Neoplastic Diseases of the  
15 Blood, Vol 1., Wiernik, P. H. et al. eds., 161-182 (1985)). Neoplasias are now believed to result from the qualitative alteration of a normal cellular gene product, or from the quantitative modification of gene expression by insertion into the chromosome of a viral sequence, by chromosomal translocation of a gene to a more actively transcribed region, or by some other mechanism. (Germann et al., supra) It  
20 is likely that mutated or altered expression of specific genes is involved in the pathogenesis of some leukemias, among other tissues and cell types. (Germann et al., supra) Indeed, the human counterparts of the oncogenes involved in some animal neoplasias have been amplified or translocated in some cases of human leukemia and carcinoma. (Germann et al., supra)

25 For example, c-myc expression is highly amplified in the non-lymphocytic leukemia cell line HL-60. When HL-60 cells are chemically induced to stop proliferation, the level of c-myc is found to be downregulated. (International Publication Number WO 91/15580) However, it has been shown that exposure of HL-60 cells to a DNA construct that is complementary to the 5' end of c-myc or c-myb blocks translation of  
30 the corresponding mRNAs which downregulates expression of the c-myc or c-myb proteins and causes arrest of cell proliferation and differentiation of the treated cells.

(International Publication Number WO 91/15580; Wickstrom et al., Proc. Natl. Acad. Sci. 85:1028 (1988); Anfossi et al., Proc. Natl. Acad. Sci. 86:3379 (1989)). However, the skilled artisan would appreciate the present invention's usefulness would not be limited to treatment of proliferative disorders of hematopoietic cells and tissues, in  
5 light of the numerous cells and cell types of varying origins which are known to exhibit proliferative phenotypes.

In addition to the foregoing, a polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991);  
10 "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). Both methods rely on binding of the polynucleotide to a complementary DNA or RNA. For these techniques, preferred  
15 polynucleotides are usually oligonucleotides 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991) ) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense  
20 Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an  
25 effort to treat disease.

Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate  
30 manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.



The polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more  
5 restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

10 The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a  
15 unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological  
20 samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum or surfactant, urine, fecal matter, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals. (Erlich, H., PCR  
25 Technology, Freeman and Co. (1992).) Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, polynucleotides of the present invention can be used as polymorphic markers for forensic purposes.

30 There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with

tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from the sequences of the present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures  
5 for contamination.

In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making  
10 oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

#### Uses of the Polypeptides

15 Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

A polypeptide of the present invention can be used to assay protein levels in a biological sample using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods. (Jalkanen, M.,  
20 et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell. Biol. 105:3087-3096 (1987).) Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such  
25 as iodine ( $^{125}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{112}\text{In}$ ), and technetium ( $^{99\text{m}}\text{Tc}$ ), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying secreted protein levels in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo  
30 imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which

emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

5       A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example,  $^{131}\text{I}$ ,  $^{112}\text{In}$ ,  $^{99\text{m}}\text{Tc}$ ), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the  
10       subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99\text{m}}\text{Tc}$ . The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein.  
15       In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).)

      Thus, the invention provides a diagnostic method of a disorder, which  
20       involves (a) assaying the expression of a polypeptide of the present invention in cells or body fluid of an individual; (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a disorder. With respect to cancer, the presence of a relatively high  
25       amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the  
30       development or further progression of the cancer.

Moreover, polypeptides of the present invention can be used to treat disease. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S  
5 for hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor suppressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel  
10 growth inhibition, enhancement of the immune response to proliferative cells or tissues).

Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat disease. For example, administration of an antibody directed to a polypeptide of the present invention can bind and reduce overproduction of the  
15 polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also  
20 be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

### Gene Therapy Methods

Another aspect of the present invention is to gene therapy methods for treating disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of a polypeptide of the present invention. This method requires a polynucleotide which codes for a polypeptide of the invention that operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a polynucleotide of the invention *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, see Belldegrin et al., J. Natl. Cancer Inst., 85:207-216 (1993); Ferrantini et al., Cancer Research, 53:107-1112 (1993); Ferrantini et al., J. Immunology 153: 4604-4615 (1994); Kaido, T., et al., Int. J. Cancer 60: 221-229 (1995); Ogura et al., Cancer Research 50: 5102-5106 (1990); Santodonato, et al., Human Gene Therapy 7:1-10 (1996); Santodonato, et al., Gene Therapy 4:1246-1255 (1997); and Zhang, et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

In one embodiment, the polynucleotide of the invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry

into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

The polynucleotide vector constructs of the invention used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

Any strong promoter known to those skilled in the art can be used for driving the expression of polynucleotide sequence of the invention. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the b-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the polynucleotides of the invention.

Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct of the invention can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney,

gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular, fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked *nucleic acid* sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

In certain embodiments, the polynucleotide constructs of the invention are  
5 complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate  
10 intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA , 84:7413-7416 (1987), which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA , 86:6077-6081 (1989), which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem., 265:10189-10192 (1990), which is herein incorporated by reference), in functional  
15 form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl Acad. Sci. USA ,  
20 84:7413-7416 (1987), which is herein incorporated by reference). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication NO: WO 90/11092  
25 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., Felgner et al., Proc. Natl. Acad. Sci. USA, 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

30 Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily



available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., *Methods of Immunology*, 101:512-527 (1983), which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated. SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and

then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods  
5 include  $\text{Ca}^{2+}$ -EDTA chelation (Papahadjopoulos et al., Biochim. Biophys. Acta, 394:483 (1975); Wilson et al., Cell, 17:77 (1979)); ether injection (Deamer et al., Biochim. Biophys. Acta, 443:629 (1976); Ostro et al., Biochem. Biophys. Res. Commun., 76:836 (1977); Fraley et al., Proc. Natl. Acad. Sci. USA, 76:3348 (1979)); detergent dialysis (Enoch et al., Proc. Natl. Acad. Sci. USA, 76:145 (1979)); and  
10 reverse-phase evaporation (REV) (Fraley et al., J. Biol. Chem., 255:10431 (1980); Szoka et al., Proc. Natl. Acad. Sci. USA, 75:145 (1978); Schaefer-Ridder et al., Science, 215:166 (1982)), which are herein incorporated by reference.

Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ratio will be from about 5:1 to about 1:5. More preferably, the  
15 ratio will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

U.S. Patent NO: 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469  
20 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide methods for delivering DNA-cationic lipid complexes to mammals.

25 In certain embodiments, cells are engineered, *ex vivo* or *in vivo*, using a retroviral particle containing RNA which comprises a sequence encoding polypeptides of the invention. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis  
30 virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy , 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO<sub>4</sub> precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding polypeptides of the invention. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express polypeptides of the invention.

In certain other embodiments, cells are engineered, *ex vivo* or *in vivo*, with polynucleotides of the invention contained in an adenovirus vector. Adenovirus can be manipulated such that it encodes and expresses polypeptides of the invention, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartz et al., Am. Rev. Respir. Dis., 109:233-238 (1974)). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld et al., Science , 252:431-434 (1991); Rosenfeld et al., Cell, 68:143-155 (1992)). Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green et al. Proc. Natl. Acad. Sci. USA , 76:6606 (1979)).

Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel., 3:499-503 (1993); Rosenfeld et al., Cell , 68:143-155 (1992); Engelhardt et al., Human Genet. Ther.,

4:759-769 (1993); Yang et al., Nature Genet., 7:362-369 (1994); Wilson et al., Nature, 365:691-692 (1993); and U.S. Patent NO: 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and  
5 constitutively express E1a and E1b, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

Preferably, the adenoviruses used in the present invention are replication  
10 deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b,  
15 E3, E4, E2a, or L1 through L5.

In certain other embodiments, the cells are engineered, *ex vivo* or *in vivo*, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, Curr. Topics in Microbiol. Immunol., 158:97 (1992)). It is also one of the few viruses that  
20 may integrate its DNA into non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

25 For example, an appropriate AAV vector for use in the present invention will include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct containing polynucleotides of the invention is inserted into the AAV vector using standard cloning methods, such as those found in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor  
30 Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper virus, using any standard technique, including

lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the polynucleotide construct of the invention.

- 5 These viral particles are then used to transduce eukaryotic cells, either *ex vivo* or *in vivo*. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express the desired gene product.

- Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding the
- 10 polypeptide sequence of interest) via homologous recombination (see, e.g., U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA, 86:8932-8935 (1989); and Zijlstra et al., Nature, 342:435-438 (1989). This method
- 15 involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

- Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently
- 20 complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5' end of the desired endogenous polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

- 25 The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the
- 30 amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can  
5 be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such  
10 that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

The polynucleotides encoding polypeptides of the present invention may be administered along with other polynucleotides encoding other angiogenic proteins. Angiogenic proteins include, but are not limited to, acidic and basic fibroblast growth  
15 factors, VEGF-1, VEGF-2 (VEGF-C), VEGF-3 (VEGF-B), epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

20 Preferably, the polynucleotide encoding a polypeptide of the invention contains a secretory signal sequence that facilitates secretion of the protein. Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5' end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be  
25 homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct  
30 needle injection, systemic injection, catheter infusion, biolistic injectors, particle accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available

depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppositorial solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the rat livers. (Kaneda et al., Science, 243:375 (1989)).

A preferred method of local administration is by direct injection. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of arteries.

Administration of a composition locally within the area of arteries refers to injecting the composition centimeters and preferably, millimeters within arteries.

Another method of local administration is to contact a polynucleotide construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue inside the wound.

Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site.

Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA, 189:11277-11281 (1992), which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a

polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise condition requiring treatment and its severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as the amount of polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian. Therapeutic compositions of the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly

#### 15 Biological Activities

The polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used in assays to test for one or more biological activities. If these polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides or polypeptides, or agonists or antagonists could be used to treat the associated disease.

#### Immune Activity

The polynucleotides or polypeptides, or agonists or antagonists of the present invention may be useful in treating deficiencies or disorders of the immune system, by activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune deficiencies or disorders may be genetic, somatic, such as cancer or some autoimmune disorders, acquired (e.g., by chemotherapy or toxins),



or infectious. Moreover, a polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

5 A polynucleotides or polypeptides, or agonists or antagonists of the present invention may be useful in treating or detecting deficiencies or disorders of hematopoietic cells. A polynucleotides or polypeptides, or agonists or antagonists of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat those disorders associated with a decrease in certain (or many) types hematopoietic cells.

10 Examples of immunologic deficiency syndromes include, but are not limited to: blood protein disorders (e.g. agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, Digeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined  
15 immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

Moreover, a polynucleotides or polypeptides, or agonists or antagonists of the present invention could also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing  
20 hemostatic or thrombolytic activity, a polynucleotides or polypeptides, or agonists or antagonists of the present invention could be used to treat blood coagulation disorders (e.g., afibrinogenemia, factor deficiencies), blood platelet disorders (e.g. thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, a polynucleotides or polypeptides, or agonists or antagonists of the  
25 present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in the treatment of heart attacks (infarction), strokes, or scarring.

A polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be useful in treating or detecting autoimmune disorders. Many  
30 autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response

leading to the destruction of the host tissue. Therefore, the administration of a polynucleotides or polypeptides, or agonists or antagonists of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

Examples of autoimmune disorders that can be treated or detected by the present invention include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disease.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated by a polynucleotides or polypeptides, or agonists or antagonists of the present invention. Moreover, these molecules can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

A polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be used to treat and/or prevent organ rejection or graft-versus-host disease (GVHD). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. The administration of a polynucleotides or polypeptides, or agonists or antagonists of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, a polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be used to modulate inflammation. For example, the polypeptide or polynucleotide or agonists

or antagonist may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat inflammatory conditions, both chronic and acute conditions, including inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

#### 10 **Hyperproliferative Disorders**

A polynucleotides or polypeptides, or agonists or antagonists of the invention can be used to treat or detect hyperproliferative disorders, including neoplasms. A polynucleotides or polypeptides, or agonists or antagonists of the present invention may inhibit the proliferation of the disorder through direct or indirect interactions.

15 Alternatively, a polynucleotides or polypeptides, or agonists or antagonists of the present invention may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative disorders can be treated. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

25 Examples of hyperproliferative disorders that can be treated or detected by a polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to neoplasms located in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative disorders can also be treated or detected by a polynucleotides or polypeptides, or agonists or antagonists of the present invention. Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstrom's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

One preferred embodiment utilizes polynucleotides of the present invention to inhibit aberrant cellular division, by gene therapy using the present invention, and/or protein fusions or fragments thereof.

Thus, the present invention provides a method for treating cell proliferative disorders by inserting into an abnormally proliferating cell a polynucleotide of the present invention, wherein said polynucleotide represses said expression.

Another embodiment of the present invention provides a method of treating cell-proliferative disorders in individuals comprising administration of one or more active gene copies of the present invention to an abnormally proliferating cell or cells. In a preferred embodiment, polynucleotides of the present invention is a DNA construct comprising a recombinant expression vector effective in expressing a DNA sequence encoding said polynucleotides. In another preferred embodiment of the present invention, the DNA construct encoding the poynucleotides of the present invention is inserted into cells to be treated utilizing a retrovirus, or more preferably an adenoviral vector (See G J. Nabel, et. al., PNAS 1999 96: 324-326, which is hereby incorporated by reference). In a most preferred embodiment, the viral vector is defective and will not transform non-proliferating cells, only proliferating cells. Moreover, in a preferred embodiment, the polynucleotides of the present invention inserted into proliferating cells either alone, or in combination with or fused to other polynucleotides, can then be modulated via an external stimulus (i.e. magnetic, specific small molecule, chemical, or drug administration, etc.), which acts upon the promoter upstream of said polynucleotides to induce expression of the encoded protein product. As such the beneficial therapeutic affect of the present invention

may be expressly modulated (i.e. to increase, decrease, or inhibit expression of the present invention) based upon said external stimulus.

Polynucleotides of the present invention may be useful in repressing expression of oncogenic genes or antigens. By "repressing expression of the oncogenic genes " is intended the suppression of the transcription of the gene, the  
5 degradation of the gene transcript (pre-message RNA), the inhibition of splicing, the destruction of the messenger RNA, the prevention of the post-translational modifications of the protein, the destruction of the protein, or the inhibition of the normal function of the protein.

10 For local administration to abnormally proliferating cells, polynucleotides of the present invention may be administered by any method known to those of skill in the art including, but not limited to transfection, electroporation, microinjection of cells, or in vehicles such as liposomes, lipofectin, or as naked polynucleotides, or any other method described throughout the specification. The polynucleotide of the  
15 present invention may be delivered by known gene delivery systems such as, but not limited to, retroviral vectors (Gilboa, J. Virology 44:845 (1982); Hocke, Nature 320:275 (1986); Wilson, et al., Proc. Natl. Acad. Sci. U.S.A. 85:3014), vaccinia virus system (Chakrabarty et al., Mol. Cell Biol. 5:3403 (1985) or other efficient DNA delivery systems (Yates et al., Nature 313:812 (1985)) known to those skilled in the  
20 art. These references are exemplary only and are hereby incorporated by reference. In order to specifically deliver or transfect cells which are abnormally proliferating and spare non-dividing cells, it is preferable to utilize a retrovirus, or adenoviral (as described in the art and elsewhere herein) delivery system known to those of skill in the art. Since host DNA replication is required for retroviral DNA to integrate and  
25 the retrovirus will be unable to self replicate due to the lack of the retrovirus genes needed for its life cycle. Utilizing such a retroviral delivery system for polynucleotides of the present invention will target said gene and constructs to abnormally proliferating cells and will spare the non-dividing normal cells.

The polynucleotides of the present invention may be delivered directly to cell  
30 proliferative disorder/disease sites in internal organs, body cavities and the like by use of imaging devices used to guide an injecting needle directly to the disease site. The

polynucleotides of the present invention may also be administered to disease sites at the time of surgical intervention.

By "cell proliferative disease" is meant any human or animal disease or disorder, affecting any one or any combination of organs, cavities, or body parts, which is characterized by single or multiple local abnormal proliferations of cells, groups of cells, or tissues, whether benign or malignant.

Any amount of the polynucleotides of the present invention may be administered as long as it has a biologically inhibiting effect on the proliferation of the treated cells. Moreover, it is possible to administer more than one of the polynucleotide of the present invention simultaneously to the same site. By "biologically inhibiting" is meant partial or total growth inhibition as well as decreases in the rate of proliferation or growth of the cells. The biologically inhibitory dose may be determined by assessing the effects of the polynucleotides of the present invention on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell cultures, or any other method known to one of ordinary skill in the art.

The present invention is further directed to antibody-based therapies which involve administering of anti-polypeptides and anti-polynucleotide antibodies to a mammalian, preferably human, patient for treating one or more of the described disorders. Methods for producing anti-polypeptides and anti-polynucleotide antibodies polyclonal and monoclonal antibodies are described in detail elsewhere herein. Such antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

In particular, the antibodies, fragments and derivatives of the present invention are useful for treating a subject having or developing cell proliferative and/or differentiation disorders as described herein. Such treatment comprises administering a single or multiple doses of the antibody, or a fragment, derivative, or a conjugate thereof.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors, for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides, including fragments thereof. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-6}M$ ,  $10^{-6}M$ ,  $5 \times 10^{-7}M$ ,  $10^{-7}M$ ,  $5 \times 10^{-8}M$ ,  $10^{-8}M$ ,  $5 \times 10^{-9}M$ ,  $10^{-9}M$ ,  $5 \times 10^{-10}M$ ,  $10^{-10}M$ ,  $5 \times 10^{-11}M$ ,  $10^{-11}M$ ,  $5 \times 10^{-12}M$ ,  $10^{-12}M$ ,  $5 \times 10^{-13}M$ ,  $10^{-13}M$ ,  $5 \times 10^{-14}M$ ,  $10^{-14}M$ ,  $5 \times 10^{-15}M$ , and  $10^{-15}M$ .

Moreover, polypeptides of the present invention are useful in inhibiting the angiogenesis of proliferative cells or tissues, either alone, as a protein fusion, or in combination with other polypeptides directly or indirectly, as described elsewhere herein. In a most preferred embodiment, said anti-angiogenesis effect may be achieved indirectly, for example, through the inhibition of hematopoietic, tumor-specific cells, such as tumor-associated macrophages (See Joseph IB, et al. J Natl Cancer Inst, 90(21):1648-53 (1998), which is hereby incorporated by reference). Antibodies directed to polypeptides or polynucleotides of the present invention may also result in inhibition of angiogenesis directly, or indirectly (See Witte L, et al., Cancer Metastasis Rev. 17(2):155-61 (1998), which is hereby incorporated by reference)).

Polypeptides, including protein fusions, of the present invention, or fragments thereof may be useful in inhibiting proliferative cells or tissues through the induction of apoptosis. Said polypeptides may act either directly, or indirectly to induce apoptosis of proliferative cells and tissues, for example in the activation of a death-domain receptor, such as tumor necrosis factor (TNF) receptor-1, CD95 (Fas/APO-1),  
5 TNF-receptor-related apoptosis-mediated protein (TRAMP) and TNF-related apoptosis-inducing ligand (TRAIL) receptor-1 and -2 (See Schulze-Osthoff K, et.al., Eur J Biochem 254(3):439-59 (1998), which is hereby incorporated by reference). Moreover, in another preferred embodiment of the present invention, said  
10 polypeptides may induce apoptosis through other mechanisms, such as in the activation of other proteins which will activate apoptosis, or through stimulating the expression of said proteins, either alone or in combination with small molecule drugs or adjuvants, such as apoptonin, galectins, thioredoxins, antiinflammatory proteins (See for example, Mutat Res 400(1-2):447-55 (1998), Med Hypotheses.50(5):423-33  
15 (1998), Chem Biol Interact. Apr 24;111-112:23-34 (1998), J Mol Med.76(6):402-12 (1998), Int J Tissue React;20(1):3-15 (1998), which are all hereby incorporated by reference).

Polypeptides, including protein fusions to, or fragments thereof, of the present invention are useful in inhibiting the metastasis of proliferative cells or tissues.  
20 Inhibition may occur as a direct result of administering polypeptides, or antibodies directed to said polypeptides as described elsewhere herein, or indirectly, such as activating the expression of proteins known to inhibit metastasis, for example alpha 4 integrins, (See, e.g., Curr Top Microbiol Immunol 1998;231:125-41, which is hereby incorporated by reference). Such therapeutic affects of the present invention may be  
25 achieved either alone, or in combination with small molecule drugs or adjuvants.

In another embodiment, the invention provides a method of delivering compositions containing the polypeptides of the invention (e.g., compositions containing polypeptides or polypeptide antibodies associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs) to targeted cells  
30 expressing the polypeptide of the present invention. Polypeptides or polypeptide antibodies of the invention may be associated with with heterologous polypeptides,



heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions.

Polypeptides, protein fusions to, or fragments thereof, of the present invention are useful in enhancing the immunogenicity and/or antigenicity of proliferating cells or tissues, either directly, such as would occur if the polypeptides of the present invention 'vaccinated' the immune response to respond to proliferative antigens and immunogens, or indirectly, such as in activating the expression of proteins known to enhance the immune response (e.g. chemokines), to said antigens and immunogens.

#### 10        Cardiovascular Disorders

Polynucleotides or polypeptides, or agonists or antagonists of the invention may be used to treat cardiovascular disorders, including peripheral artery disease, such as limb ischemia.

Cardiovascular disorders include cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome. Congenital heart defects include aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia, patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects, Lutembacher's Syndrome, trilogly of Fallot, ventricular heart septal defects.

Cardiovascular disorders also include heart disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right ventricular hypertrophy, post-infarction heart rupture, ventricular septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease,

ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.

Arrhythmias include sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial  
5 block, long QT syndrome, parasystole, Lown-Ganong-Levine Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, tachycardias, and ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic  
10 junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia, Torsades de Pointes, and ventricular tachycardia.

Heart valve disease include aortic valve insufficiency, aortic valve stenosis, hear murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve  
15 insufficiency, pulmonary valve stenosis, tricuspid atresia, tricuspid valve insufficiency, and tricuspid valve stenosis.

Myocardial diseases include alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular stenosis, pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial  
20 reperfusion injury, and myocarditis.

Myocardial ischemias include coronary disease, such as angina pectoris, coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial infarction and myocardial stunning.

25 Cardiovascular diseases also include vascular diseases such as aneurysms, angiodysplasia, angiomas, bacillary angiomas, Hippel-Lindau Disease, Klippel-Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema, aortic diseases, Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive diseases, arteritis, enarteritis, polyarteritis nodosa, cerebrovascular disorders, diabetic  
30 angiopathies, diabetic retinopathy, embolisms, thrombosis, erythromelalgia, hemorrhoids, hepatic veno-occlusive disease, hypertension, hypotension, ischemia,

peripheral vascular diseases, phlebitis, pulmonary veno-occlusive disease, Raynaud's disease, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, atacia telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose ulcer, vasculitis, and venous  
5 insufficiency.

Aneurysms include dissecting aneurysms, false aneurysms, infected aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart aneurysms, and iliac aneurysms.

Arterial occlusive diseases include arteriosclerosis, intermittent claudication,  
10 carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal artery obstruction, retinal artery occlusion, and thromboangiitis obliterans.

Cerebrovascular disorders include carotid artery diseases, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral  
15 arteriovenous malformation, cerebral artery diseases, cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural hematoma, subarachnoid hemorrhage, cerebral infarction, cerebral ischemia (including transient), subclavian steal syndrome, periventricular leukomalacia, vascular headache, cluster headache,  
20 migraine, and vertebrobasilar insufficiency.

Embolisms include air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and thromboembolisms. Thrombosis include coronary thrombosis, hepatic vein thrombosis, retinal vein occlusion, carotid artery thrombosis, sinus thrombosis,  
25 Wallenberg's syndrome, and thrombophlebitis.

Ischemia includes cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion injuries, and peripheral limb ischemia. Vasculitis includes aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome,  
30 thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and Wegener's granulomatosis.

Polynucleotides or polypeptides, or agonists or antagonists of the invention, are especially effective for the treatment of critical limb ischemia and coronary disease.

5 Polypeptides may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, biolistic injectors, particle accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppository solid pharmaceutical formulations, decanting or  
10 art. Polypeptides of the invention may be administered as part of a *Therapeutic*, described in more detail below. Methods of delivering polynucleotides of the invention are described in more detail herein.

#### Anti-Angiogenesis Activity

15 The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences predominate. Rastinejad *et al.*, *Cell* 56:345-355 (1989). In those rare instances in which neovascularization occurs under normal physiological conditions, such as wound healing, organ regeneration, embryonic development, and female reproductive  
20 processes, angiogenesis is stringently regulated and spatially and temporally delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated angiogenesis becomes pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number of serious diseases are dominated by abnormal neovascularization  
25 including solid tumor growth and metastases, arthritis, some types of eye disorders, and psoriasis. See, e.g., reviews by Moses *et al.*, *Biotech.* 9:630-634 (1991); Folkman *et al.*, *N. Engl. J. Med.*, 333:1757-1763 (1995); Auerbach *et al.*, *J. Microvasc. Res.* 29:401-411 (1985); Folkman, *Advances in Cancer Research*, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, *Am. J.*  
30 *Ophthalmol.* 94:715-743 (1982); and Folkman *et al.*, *Science* 221:719-725 (1983). In a number of pathological conditions, the process of angiogenesis contributes to the

disease state. For example, significant data have accumulated which suggest that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun, *Science* 235:442-447 (1987).

The present invention provides for treatment of diseases or disorders  
5 associated with neovascularization by administration of the polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists of the present invention. Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides, or agonists or antagonists of the invention include, but are not limited to, malignancies, solid tumors, and cancers described herein and  
10 otherwise known in the art (for a review of such disorders, see Fishman *et al.*, Medicine, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)). Thus, the present invention provides a method of treating an angiogenesis-related disease and/or disorder, comprising administering to an individual in need thereof a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist of the  
15 invention. For example, polynucleotides, polypeptides, antagonists and/or agonists may be utilized in a variety of additional methods in order to therapeutically treat a cancer or tumor. Cancers which may be treated with polynucleotides, polypeptides, antagonists and/or agonists include, but are not limited to solid tumors, including prostate, lung, breast, ovarian, stomach, pancreas, larynx, esophagus, testes, liver,  
20 parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder, thyroid cancer; primary tumors and metastases; melanomas; glioblastoma; Kaposi's sarcoma; leiomyosarcoma; non-small cell lung cancer; colorectal cancer; advanced malignancies; and blood born tumors such as leukemias. For example, polynucleotides, polypeptides, antagonists and/or agonists may be delivered topically,  
25 in order to treat cancers such as skin cancer, head and neck tumors, breast tumors, and Kaposi's sarcoma.

Within yet other aspects, polynucleotides, polypeptides, antagonists and/or agonists may be utilized to treat superficial forms of bladder cancer by, for example, intravesical administration. Polynucleotides, polypeptides, antagonists and/or agonists  
30 may be delivered directly into the tumor, or near the tumor site, via injection or a catheter. Of course, as the artisan of ordinary skill will appreciate, the appropriate

mode of administration will vary according to the cancer to be treated. Other modes of delivery are discussed herein.

Polynucleotides, polypeptides, antagonists and/or agonists may be useful in treating other disorders, besides cancers, which involve angiogenesis. These disorders include, but are not limited to: benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; arteriosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophilic joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis.

For example, within one aspect of the present invention methods are provided for treating hypertrophic scars and keloids, comprising the step of administering a polynucleotide, polypeptide, antagonist and/or agonist of the invention to a hypertrophic scar or keloid.

Within one embodiment of the present invention polynucleotides, polypeptides, antagonists and/or agonists are directly injected into a hypertrophic scar or keloid, in order to prevent the progression of these lesions. This therapy is of particular value in the prophylactic treatment of conditions which are known to result in the development of hypertrophic scars and keloids (e.g., burns), and is preferably initiated after the proliferative phase has had time to progress (approximately 14 days after the initial injury), but before hypertrophic scar or keloid development. As noted above, the present invention also provides methods for treating neovascular diseases of the eye, including for example, corneal neovascularization, neovascular glaucoma, proliferative diabetic retinopathy, retrolental fibroplasia and macular degeneration.

Moreover, Ocular disorders associated with neovascularization which can be

treated with the polynucleotides and polypeptides of the present invention (including agonists and/or antagonists) include, but are not limited to: neovascular glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity macular degeneration, corneal graft neovascularization, as well as other  
5 eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.g., reviews by Waltman *et al.*, *Am. J. Ophthalmol.* 85:704-710 (1978) and Gartner *et al.*, *Surv. Ophthalmol.* 22:291-312 (1978).

Thus, within one aspect of the present invention methods are provided for treating neovascular diseases of the eye such as corneal neovascularization (including  
10 corneal graft neovascularization), comprising the step of administering to a patient a therapeutically effective amount of a compound (as described above) to the cornea, such that the formation of blood vessels is inhibited. Briefly, the cornea is a tissue which normally lacks blood vessels. In certain pathological conditions however, capillaries may extend into the cornea from the pericorneal vascular plexus of the  
15 limbus. When the cornea becomes vascularized, it also becomes clouded, resulting in a decline in the patient's visual acuity. Visual loss may become complete if the cornea completely opacitates. A wide variety of disorders can result in corneal neovascularization, including for example, corneal infections (e.g., trachoma, herpes simplex keratitis, leishmaniasis and onchocerciasis), immunological processes (e.g.,  
20 graft rejection and Stevens-Johnson's syndrome), alkali burns, trauma, inflammation (of any cause), toxic and nutritional deficiency states, and as a complication of wearing contact lenses.

Within particularly preferred embodiments of the invention, may be prepared for topical administration in saline (combined with any of the preservatives and  
25 antimicrobial agents commonly used in ocular preparations), and administered in eyedrop form. The solution or suspension may be prepared in its pure form and administered several times daily. Alternatively, anti-angiogenic compositions, prepared as described above, may also be administered directly to the cornea. Within preferred embodiments, the anti-angiogenic composition is prepared with a muco-  
30 adhesive polymer which binds to cornea. Within further embodiments, the anti-angiogenic factors or anti-angiogenic compositions may be utilized as an adjunct to

conventional steroid therapy. Topical therapy may also be useful prophylactically in corneal lesions which are known to have a high probability of inducing an angiogenic response (such as chemical burns). In these instances the treatment, likely in combination with steroids, may be instituted immediately to help prevent subsequent complications.

Within other embodiments, the compounds described above may be injected directly into the corneal stroma by an ophthalmologist under microscopic guidance. The preferred site of injection may vary with the morphology of the individual lesion, but the goal of the administration would be to place the composition at the advancing front of the vasculature (i.e., interspersed between the blood vessels and the normal cornea). In most cases this would involve perilimbic corneal injection to "protect" the cornea from the advancing blood vessels. This method may also be utilized shortly after a corneal insult in order to prophylactically prevent corneal neovascularization. In this situation the material could be injected in the perilimbic cornea interspersed between the corneal lesion and its undesired potential limbic blood supply. Such methods may also be utilized in a similar fashion to prevent capillary invasion of transplanted corneas. In a sustained-release form injections might only be required 2-3 times per year. A steroid could also be added to the injection solution to reduce inflammation resulting from the injection itself.

Within another aspect of the present invention, methods are provided for treating neovascular glaucoma, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. In one embodiment, the compound may be administered topically to the eye in order to treat early forms of neovascular glaucoma. Within other embodiments, the compound may be implanted by injection into the region of the anterior chamber angle. Within other embodiments, the compound may also be placed in any location such that the compound is continuously released into the aqueous humor. Within another aspect of the present invention, methods are provided for treating proliferative diabetic retinopathy, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the



eyes, such that the formation of blood vessels is inhibited.

Within particularly preferred embodiments of the invention, proliferative diabetic retinopathy may be treated by injection into the aqueous humor or the vitreous, in order to increase the local concentration of the polynucleotide, polypeptide, antagonist and/or agonist in the retina. Preferably, this treatment should  
5 be initiated prior to the acquisition of severe disease requiring photocoagulation.

Within another aspect of the present invention, methods are provided for treating retrolental fibroplasia, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or  
10 agonist to the eye, such that the formation of blood vessels is inhibited. The compound may be administered topically, via intravitreal injection and/or via intraocular implants.

Additionally, disorders which can be treated with the polynucleotides, polypeptides, agonists and/or antagonists include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber  
15 syndrome, pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

Moreover, disorders and/or states, which can be treated with the polynucleotides, polypeptides, agonists and/or antagonists include, but are not limited to, solid tumors, blood born tumors such as leukemias, tumor metastasis, Kaposi's sarcoma, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, and uveitis, delayed wound healing, endometriosis, vasculogenesis, granulations, hypertrophic scars (keloids), nonunion fractures, scleroderma, trachoma, vascular adhesions, myocardial angiogenesis, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis, Osler-Webber Syndrome, plaque neovascularization, telangiectasia, hemophilic joints, angiofibroma fibromuscular dysplasia, wound granulation, Crohn's disease, atherosclerosis, birth control agent by preventing vascularization  
20  
25  
30

required for embryo implantation controlling menstruation, diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (Rochéle minalia quintosa), ulcers (*Helicobacter pylori*), Bartonellosis and bacillary angiomatosis.

5 In one aspect of the birth control method, an amount of the compound sufficient to block embryo implantation is administered before or after intercourse and fertilization have occurred, thus providing an effective method of birth control, possibly a "morning after" method. Polynucleotides, polypeptides, agonists and/or agonists may also be used in controlling menstruation or administered as either a  
10 peritoneal lavage fluid or for peritoneal implantation in the treatment of endometriosis.

Polynucleotides, polypeptides, agonists and/or agonists of the present invention may be incorporated into surgical sutures in order to prevent stitch granulomas.

15 Polynucleotides, polypeptides, agonists and/or agonists may be utilized in a wide variety of surgical procedures. For example, within one aspect of the present invention a compositions (in the form of, for example, a spray or film) may be utilized to coat or spray an area prior to removal of a tumor, in order to isolate normal surrounding tissues from malignant tissue, and/or to prevent the spread of disease to  
20 surrounding tissues. Within other aspects of the present invention, compositions (e.g., in the form of a spray) may be delivered via endoscopic procedures in order to coat tumors, or inhibit angiogenesis in a desired locale. Within yet other aspects of the present invention, surgical meshes which have been coated with anti- angiogenic compositions of the present invention may be utilized in any procedure wherein a  
25 surgical mesh might be utilized. For example, within one embodiment of the invention a surgical mesh laden with an anti-angiogenic composition may be utilized during abdominal cancer resection surgery (e.g., subsequent to colon resection) in order to provide support to the structure, and to release an amount of the anti-angiogenic factor.

30 Within further aspects of the present invention, methods are provided for treating tumor excision sites, comprising administering a polynucleotide, polypeptide,

agonist and/or agonist to the resection margins of a tumor subsequent to excision, such that the local recurrence of cancer and the formation of new blood vessels at the site is inhibited. Within one embodiment of the invention, the anti-angiogenic compound is administered directly to the tumor excision site (e.g., applied by swabbing, brushing or otherwise coating the resection margins of the tumor with the anti-angiogenic compound). Alternatively, the anti-angiogenic compounds may be incorporated into known surgical pastes prior to administration. Within particularly preferred embodiments of the invention, the anti-angiogenic compounds are applied after hepatic resections for malignancy, and after neurosurgical operations.

Within one aspect of the present invention, polynucleotides, polypeptides, agonists and/or agonists may be administered to the resection margin of a wide variety of tumors, including for example, breast, colon, brain and hepatic tumors. For example, within one embodiment of the invention, anti-angiogenic compounds may be administered to the site of a neurological tumor subsequent to excision, such that the formation of new blood vessels at the site are inhibited.

The polynucleotides, polypeptides, agonists and/or agonists of the present invention may also be administered along with other anti-angiogenic factors. Representative examples of other anti-angiogenic factors include: Anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel, Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl

complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten  
5 oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its  
10 hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and  
15 sugars.

A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan  
20 Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin;  
25 Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, 1992); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, 1992); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, 1990); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, 1987); anticollagenase-serum;  
30 alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, 1987); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4-

chloroanthronilic acid disodium or "CCA"; Takeuchi et al., Agents Actions 36:312-316, 1992); Thalidomide; Angostatic steroid; AGM-1470; carboxynaminolmidazole; and metalloproteinase inhibitors such as BB94.

5           **Diseases at the Cellular Level**

Diseases associated with increased cell survival or the inhibition of apoptosis that could be treated or detected by the polynucleotides or polypeptides and/or antagonists or agonists of the invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. In preferred embodiments, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention are used to inhibit growth, progression, and/or metasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be treated or detected by the polynucleotides or polypeptides, or agonists or antagonists of the invention, include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy

- chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.
- 15 Diseases associated with increased apoptosis that could be treated or detected by the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

### **Wound Healing and Epithelial Cell Proliferation**

- 30 In accordance with yet a further aspect of the present invention, there is provided a process for utilizing the polynucleotides or polypeptides, and/or agonists

or antagonists of the invention, for therapeutic purposes, for example, to stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound healing, and to stimulate hair follicle production and healing of dermal wounds. Polynucleotides or polypeptides, as well as agonists or antagonists of the invention, may be clinically useful in stimulating wound healing including surgical wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis, eye tissue wounds, dental tissue wounds, oral cavity wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat exposure or chemicals, and other abnormal wound healing conditions such as uremia, malnutrition, vitamin deficiencies and complications associated with systemic treatment with steroids, radiation therapy and antineoplastic drugs and antimetabolites. Polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to promote dermal reestablishment subsequent to dermal loss

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to increase the adherence of skin grafts to a wound bed and to stimulate re-epithelialization from the wound bed. The following are a non-exhaustive list of grafts that polynucleotides or polypeptides, agonists or antagonists of the invention, could be used to increase adherence to a wound bed: autografts, artificial skin, allografts, autodermic graft, autoepidermic grafts, avascular grafts, Blair-Brown grafts, bone graft, brephoplastic grafts, cutis graft, delayed graft, dermic graft, epidermic graft, fascia graft, full thickness graft, heterologous graft, xenograft, homologous graft, hyperplastic graft, lamellar graft, mesh graft, mucosal graft, Ollier-Thiersch graft, omentum graft, patch graft, pedicle graft, penetrating graft, split skin graft, thick split graft. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, can be used to promote skin strength and to improve the appearance of aged skin.

It is believed that the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, will also produce changes in hepatocyte proliferation, and epithelial cell proliferation in the lung, breast, pancreas, stomach, small intestine, and large intestine. The polynucleotides or polypeptides, and/or agonists or

antagonists of the invention, could promote proliferation of epithelial cells such as sebocytes, hair follicles, hepatocytes, type II pneumocytes, mucin-producing goblet cells, and other epithelial cells and their progenitors contained within the skin, lung, liver, and gastrointestinal tract. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may promote proliferation of endothelial cells, keratinocytes, and basal keratinocytes.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could also be used to reduce the side effects of gut toxicity that result from radiation, chemotherapy treatments or viral infections. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may have a cytoprotective effect on the small intestine mucosa. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may also stimulate healing of mucositis (mouth ulcers) that result from chemotherapy and viral infections.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could further be used in full regeneration of skin in full and partial thickness skin defects, including burns, (i.e., repopulation of hair follicles, sweat glands, and sebaceous glands), treatment of other skin defects such as psoriasis. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to treat epidermolysis bullosa, a defect in adherence of the epidermis to the underlying dermis, which results in frequent, open and painful blisters by accelerating reepithelialization of these lesions. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could also be used to treat gastric and duodenal ulcers and help heal by scar formation of the mucosal lining and regeneration of glandular mucosa and duodenal mucosal lining more rapidly. Inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are diseases which result in destruction of the mucosal surface of the small or large intestine, respectively. Thus, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to promote the resurfacing of the mucosal surface to aid more rapid healing and to prevent progression of inflammatory bowel disease. Treatment with the polynucleotides or polypeptides, and/or agonists or



antagonists of the invention, is expected to have a significant effect on the production of mucus throughout the gastrointestinal tract and could be used to protect the intestinal mucosa from injurious substances that are ingested or following surgery. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to treat diseases associate with the under expression of the polynucleotides of the invention.

Moreover, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to prevent and heal damage to the lungs due to various pathological states. A growth factor such as the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, which could stimulate proliferation and differentiation and promote the repair of alveoli and brochiolar epithelium to prevent or treat acute or chronic lung damage. For example, emphysema, which results in the progressive loss of aveoli, and inhalation injuries, i.e., resulting from smoke inhalation and burns, that cause necrosis of the bronchiolar epithelium and alveoli could be effectively treated using the polynucleotides or polypeptides, and/or agonists or antagonists of the invention. Also, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to stimulate the proliferation of and differentiation of type II pneumocytes, which may help treat or prevent disease such as hyaline membrane diseases, such as infant respiratory distress syndrome and bronchopulmonary dysplasia, in premature infants.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could stimulate the proliferation and differentiation of hepatocytes and, thus, could be used to alleviate or treat liver diseases and pathologies such as fulminant liver failure caused by cirrhosis, liver damage caused by viral hepatitis and toxic substances (i.e., acetaminophen, carbon tetraholoride and other hepatotoxins known in the art).

In addition, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used treat or prevent the onset of diabetes mellitus. In patients with newly diagnosed Types I and II diabetes, where some islet cell function remains, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to maintain the islet function so as to alleviate, delay or

prevent permanent manifestation of the disease. Also, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used as an auxiliary in islet cell transplantation to improve or promote islet cell function.

## 5 Infectious Disease

A polypeptide or polynucleotide and/or agonist or antagonist of the present invention can be used to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, polypeptide or polynucleotide and/or agonist or antagonist of the present invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention. Examples of viruses, include, but are not limited to Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papiloma virus, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiollitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic

fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or diseases. In specific  
5   embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat patients nonresponsive to one or more other commercially available hepatitis vaccines. In a further specific embodiment  
10   polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat AIDS.

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, include, but not limited  
15   to, the following Gram-Negative and Gram-positive bacteria and bacterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), Cryptococcus neoformans, Aspergillosis, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia (e.g., Borrelia burgdorferi, Brucellosis, Candidiasis, Campylobacter, Coccidioidomycosis, Cryptococcosis,  
20   Dermatocycoses, E. coli (e.g., Enterotoxigenic E. coli and Enterohemorrhagic E. coli), Enterobacteriaceae (Klebsiella, Salmonella (e.g., Salmonella typhi, and Salmonella paratyphi), Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis, Leptospirosis, Listeria, Mycoplasmatales, Mycobacterium leprae, Vibrio cholerae, Neisseriaceae (e.g., Acinetobacter, Gonorrhea, Meningococcal),  
25   Meisseria meningitidis, Pasteurellacea Infections (e.g., Actinobacillus, Heamophilus (e.g., Heamophilus influenza type B), Pasteurella), Pseudomonas, Rickettsiaceae, Chlamydiaceae, Syphilis, Shigella spp., Staphylococcal, Meningiococcal, Pneumococcal and Streptococcal (e.g., Streptococcus pneumoniae and Group B Streptococcus). These bacterial or fungal families can cause the following diseases or  
30   symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS

related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis (e.g., meningitis types A and B),

5 Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. Polynucleotides or polypeptides, agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or

10 diseases. In specific embodiments, Ppolynucleotides, polypeptides, agonists or antagonists of the invention are used to treat: tetanus, Diphtheria, botulism, and/or meningitis type B.

Moreover, parasitic agents causing disease or symptoms that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the

15 present invention include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g., Plasmodium virax, Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale). These

20 parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat or detect any of these

25 symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat malaria.

Preferably, treatment using a polypeptide or polynucleotide and/or agonist or antagonist of the present invention could either be by administering an effective amount of a polypeptide to the patient, or by removing cells from the patient,

30 supplying the cells with a polynucleotide of the present invention, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the polypeptide or

polynucleotide of the present invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

### Regeneration

5 A polynucleotide or polypeptide and/or agonist or antagonist of the present invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997).) The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

10 Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vasculature (including vascular and lymphatics), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

15 Moreover, a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage.

20 A polynucleotide or polypeptide and/or agonist or antagonist of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

25 Similarly, nerve and brain tissue could also be regenerated by using a polynucleotide or polypeptide and/or agonist or antagonist of the present invention to proliferate and differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with peripheral

nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated using the polynucleotide or polypeptide and/or agonist or antagonist of the present invention.

### **Chemotaxis**

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention may have chemotaxis activity. A chemotactic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase chemotactic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotactic molecules can be used to treat wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat wounds.

It is also contemplated that a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may inhibit chemotactic activity. These molecules could also be used to treat disorders. Thus, a polynucleotide or polypeptide and/or agonist or antagonist of the present invention could be used as an inhibitor of chemotaxis.

### **Binding Activity**

A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The

binding of the polypeptide and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

- 5            Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991).) Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or at least, a fragment of the receptor capable  
10 of being bound by the polypeptide (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

- Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, *Drosophila*, or *E. coli*.  
15 Cells expressing the polypeptide (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

- The assay may simply test binding of a candidate compound to the  
20 polypeptide, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

- Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product  
25 mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

- Preferably, an ELISA assay can measure polypeptide level or activity in a  
30 sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The

antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

Additionally, the receptor to which a polypeptide of the invention binds can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan, et al., Current Protocols in Immun., 1(2), Chapter 5, (1991)). For example, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the polypeptides, for example, NIH3T3 cells which are known to contain multiple receptors for the FGF family proteins, and SC-3 cells, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the polypeptides. Transfected cells which are grown on glass slides are exposed to the polypeptide of the present invention, after they have been labelled. The polypeptides can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase.

Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an iterative sub-pooling and re-screening process, eventually yielding a single clones that encodes the putative receptor.

As an alternative approach for receptor identification, the labeled polypeptides can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE analysis and exposed to X-ray film. The labeled complex containing the receptors of the polypeptides can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

Moreover, the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of polypeptides of the invention thereby effectively generating agonists and antagonists of polypeptides of the invention. See generally, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and



5,837,458, and Patten, P. A., et al., Curr. Opinion Biotechnol. 8:724-33 (1997); Harayama, S. Trends Biotechnol. 16(2):76-82 (1998); Hansson, L. O., et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R. Biotechniques 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of polynucleotides and corresponding polypeptides of the invention may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired polynucleotide sequence of the invention molecule by homologous, or site-specific, recombination. In another embodiment, polynucleotides and corresponding polypeptides of the invention may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of the polypeptides of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are family members. In further preferred embodiments, the heterologous molecule is a growth factor such as, for example, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), transforming growth factor (TGF)-alpha, epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-beta, bone morphogenetic protein (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A and B, decapentaplegic(dpp), 60A, OP-2, dorsalin, growth differentiation factors (GDFs), nodal, MIS, inhibin-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta5, and glial-derived neurotrophic factor (GDNF).

Other preferred fragments are biologically active fragments of the polypeptides of the invention. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Additionally, this invention provides a method of screening compounds to identify those which modulate the action of the polypeptide of the present invention. An example of such an assay comprises combining a mammalian fibroblast cell, a the

polypeptide of the present invention, the compound to be screened and  $3[H]$  thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of fibroblast proliferation in the presence of the compound to determine if the compound stimulates proliferation by determining the uptake of  $3[H]$  thymidine in each case. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of  $3[H]$  thymidine. Both agonist and antagonist compounds may be identified by this procedure.

10 In another method, a mammalian cell or membrane preparation expressing a receptor for a polypeptide of the present invention is incubated with a labeled polypeptide of the present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following  
15 interaction of a compound to be screened and the receptor is measured and the ability of the compound to bind to the receptor and elicit a second messenger response is measured to determine if the compound is a potential agonist or antagonist. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

20 All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptides of the invention from  
25 suitably manipulated cells or tissues. Therefore, the invention includes a method of identifying compounds which bind to the polypeptides of the invention comprising the steps of: (a) incubating a candidate binding compound with the polypeptide; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate  
30 compound with the polypeptide, (b) assaying a biological activity, and (b) determining if a biological activity of the polypeptide has been altered.

Also, one could identify molecules bind a polypeptide of the invention experimentally by using the beta-pleated sheet regions contained in the polypeptide sequence of the protein. Accordingly, specific embodiments of the invention are directed to polynucleotides encoding polypeptides which comprise, or alternatively consist of, the amino acid sequence of each beta pleated sheet regions in a disclosed polypeptide sequence. Additional embodiments of the invention are directed to polynucleotides encoding polypeptides which comprise, or alternatively consist of, any combination or all of contained in the polypeptide sequences of the invention. Additional preferred embodiments of the invention are directed to polypeptides which comprise, or alternatively consist of, the amino acid sequence of each of the beta pleated sheet regions in one of the polypeptide sequences of the invention. Additional embodiments of the invention are directed to polypeptides which comprise, or alternatively consist of, any combination or all of the beta pleated sheet regions in one of the polypeptide sequences of the invention.

15

### **Targeted Delivery**

In another embodiment, the invention provides a method of delivering compositions to targeted cells expressing a receptor for a polypeptide of the invention, or cells expressing a cell bound form of a polypeptide of the invention.

20

As discussed herein, polypeptides or antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (including antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

30

In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., polypeptides of the invention or antibodies of the invention) in association with toxins or cytotoxic prodrugs.

5 By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes  
10 known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. By "cytotoxic prodrug" is meant a  
15 non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives  
20 of doxorubicin.

#### Drug Screening

Further contemplated is the use of the polypeptides of the present invention, or the polynucleotides encoding these polypeptides, to screen for molecules which  
25 modify the activities of the polypeptides of the present invention. Such a method would include contacting the polypeptide of the present invention with a selected compound(s) suspected of having antagonist or agonist activity, and assaying the activity of these polypeptides following binding.

This invention is particularly useful for screening therapeutic compounds by  
30 using the polypeptides of the present invention, or binding fragments thereof, in any of a variety of drug screening techniques. The polypeptide or fragment employed in

such a test may be affixed to a solid support, expressed on a cell surface, free in solution, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed  
5 cells in competitive binding assays. One may measure, for example, the formulation of complexes between the agent being tested and a polypeptide of the present invention.

Thus, the present invention provides methods of screening for drugs or any other agents which affect activities mediated by the polypeptides of the present  
10 invention. These methods comprise contacting such an agent with a polypeptide of the present invention or a fragment thereof and assaying for the presence of a complex between the agent and the polypeptide or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically labeled. Following incubation, free agent is separated from that present in bound  
15 form, and the amount of free or uncomplexed label is a measure of the ability of a particular agent to bind to the polypeptides of the present invention.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the polypeptides of the present invention, and is described in great detail in European Patent Application 84/03564,  
20 published on September 13, 1984, which is incorporated herein by reference herein. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptides of the present invention and washed. Bound polypeptides are then detected by methods well known in the art.  
25 Purified polypeptides are coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding polypeptides of the present  
30 invention specifically compete with a test compound for binding to the polypeptides or fragments thereof. In this manner, the antibodies are used to detect the presence of

any peptide which shares one or more antigenic epitopes with a polypeptide of the invention.

### **Antisense And Ribozyme (Antagonists)**

- 5 In specific embodiments, antagonists according to the present invention are nucleic acids corresponding to the sequences contained in SEQ ID NO:X, or the complementary strand thereof, and/or to nucleotide sequences contained a deposited clone. In one embodiment, antisense sequence is generated internally by the organism, in another embodiment, the antisense sequence is separately administered
- 10 (see, for example, O'Connor, Neurochem., 56:560 (1991). Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense
- 15 Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee et al., Nucleic Acids Research, 6:3073 (1979); Cooney et al., Science, 241:456 (1988); and Dervan et al., Science, 251:1300 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.
- 20 For example, the use of c-myc and c-myb antisense RNA constructs to inhibit the growth of the non-lymphocytic leukemia cell line HL-60 and other cell lines was previously described. (Wickstrom et al. (1988); Anfossi et al. (1989)). These experiments were performed in vitro by incubating cells with the oligoribonucleotide. A similar procedure for in vivo use is described in WO 91/15580. Briefly, a pair of
- 25 oligonucleotides for a given antisense RNA is produced as follows: A sequence complimentary to the first 15 bases of the open reading frame is flanked by an EcoRI site on the 5' end and a HindIII site on the 3' end. Next, the pair of oligonucleotides is heated at 90°C for one minute and then annealed in 2X ligation buffer (20mM TRIS HCl pH 7.5, 10mM MgCl<sub>2</sub>, 10mM dithiothreitol (DTT) and 0.2 mM ATP) and then
- 30 ligated to the EcoRI/Hind III site of the retroviral vector PMV7 (WO 91/15580).

For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into receptor polypeptide.

In one embodiment, the antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the antisense nucleic acid of the invention. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding a polypeptide of the invention, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, *Nature*, 29:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell*, 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., *Proc. Natl. Acad. Sci. U.S.A.*, 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster et al., *Nature*, 296:39-42 (1982)), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene of interest. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded antisense nucleic acids of the invention, a single strand of the duplex DNA may thus be tested, or triplex formation

may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with a RNA sequence of the invention it may contain and still form a stable duplex (or triplex as the case may be).

5 One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the message, *e.g.*, the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., *Nature*, 372:333-335 (1994). Thus, oligonucleotides complementary to either the 5' - or 3' - non- translated, non-coding regions of a polynucleotide sequence of the invention could be used in an antisense approach to inhibit translation of endogenous mRNA.

15 Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5' -, 3' - or coding region of mRNA, antisense nucleic acids should be at least six

20 nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-

25 stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger et al., *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556

30 (1989); Lemaitre et al., *Proc. Natl. Acad. Sci.*, 84:648-652 (1987); PCT Publication NO: WO88/09810, published December 15, 1988) or the blood-brain barrier (see,



e.g., PCT Publication NO: WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., BioTechniques, 6:958-976 (1988)) or intercalating agents. (See, e.g., Zon, Pharm. Res., 5:539-549 (1988)). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide,  
5 hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine,  
10 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil,  
15 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v),  
20 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

25 In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

30 In yet another embodiment, the antisense oligonucleotide is an a-anomeric oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded

hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gautier et al., Nucl. Acids Res., 15:6625-6641 (1987)).

The oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., Nucl. Acids Res., 15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue et al., FEBS Lett. 215:327-330 (1987)).

Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (Nucl. Acids Res., 16:3209 (1988)), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., Proc. Natl. Acad. Sci. U.S.A., 85:7448-7451 (1988)), etc.

While antisense nucleotides complementary to the coding region sequence of the invention could be used, those complementary to the transcribed untranslated region are most preferred.

Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, Science, 247:1222-1225 (1990)). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs corresponding to the polynucleotides of the invention, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5' -UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature, 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within each nucleotide sequence disclosed in the sequence listing. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the mRNA corresponding to the polynucleotides of the invention; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express the polynucleotides of the invention in vivo. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Antagonist/agonist compounds may be employed to inhibit the cell growth and proliferation effects of the polypeptides of the present invention on neoplastic cells and tissues, i.e. stimulation of angiogenesis of tumors, and, therefore, retard or prevent abnormal cellular growth and proliferation, for example, in tumor formation or growth.

The antagonist/agonist may also be employed to prevent hyper-vascular diseases, and prevent the proliferation of epithelial lens cells after extracapsular cataract surgery. Prevention of the mitogenic activity of the polypeptides of the present invention may also be desirable in cases such as restenosis after balloon angioplasty.

The antagonist/agonist may also be employed to prevent the growth of scar tissue during wound healing.

The antagonist/agonist may also be employed to treat the diseases described herein.

Thus, the invention provides a method of treating disorders or diseases, including but not limited to the disorders or diseases listed throughout this application, associated with overexpression of a polynucleotide of the present invention by administering to a patient (a) an antisense molecule directed to the polynucleotide of the present invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention

### Other Activities

The polypeptide of the present invention, as a result of the ability to stimulate vascular endothelial cell growth, may be employed in treatment for stimulating re-vascularization of ischemic tissues due to various disease conditions such as thrombosis, arteriosclerosis, and other cardiovascular conditions. These polypeptide  
5 may also be employed to stimulate angiogenesis and limb regeneration, as discussed above.

The polypeptide may also be employed for treating wounds due to injuries, burns, post-operative tissue repair, and ulcers since they are mitogenic to various cells  
10 of different origins, such as fibroblast cells and skeletal muscle cells, and therefore, facilitate the repair or replacement of damaged or diseased tissue.

The polypeptide of the present invention may also be employed stimulate neuronal growth and to treat and prevent neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions such as Alzheimer's disease,  
15 Parkinson's disease, and AIDS-related complex. The polypeptide of the invention may have the ability to stimulate chondrocyte growth, therefore, they may be employed to enhance bone and periodontal regeneration and aid in tissue transplants or bone grafts.

The polypeptide of the present invention may be also be employed to prevent  
20 skin aging due to sunburn by stimulating keratinocyte growth.

The polypeptide of the invention may also be employed for preventing hair loss, since FGF family members activate hair-forming cells and promotes melanocyte growth. Along the same lines, the polypeptides of the present invention may be employed to stimulate growth and differentiation of hematopoietic cells and bone  
25 marrow cells when used in combination with other cytokines.

The polypeptide of the invention may also be employed to maintain organs before transplantation or for supporting cell culture of primary tissues.

The polypeptide of the present invention may also be employed for inducing tissue of mesodermal origin to differentiate in early embryos.

The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

5 The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, polypeptides or polynucleotides and/or agonist or antagonists of the present invention may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing,  
10 utilization, and storage of energy.

Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may be used to change a mammal's mental state or physical state by influencing biorhythms, cardiac rhythms, depression (including depressive disorders), tendency for violence, tolerance for pain, reproductive capabilities  
15 (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins,  
20 minerals, cofactors or other nutritional components.

### **Other Preferred Embodiments**

Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95%  
25 identical to a sequence of at least about 50 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of

the Clone Sequence and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the Start Codon and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Similarly preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the First Amino Acid of the Signal Peptide and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

Further preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 500 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

A further preferred embodiment is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ ID NO:X beginning with the nucleotide at about the position of the 5' Nucleotide of the First Amino Acid of the Signal Peptide and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NO:X.

Also preferred is an isolated nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization

conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

Also preferred is a composition of matter comprising a DNA molecule which comprises a human cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in the material deposited with the American Type Culture Collection and given the ATCC Deposit Number shown in Table 1 for said cDNA Clone Identifier.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in the nucleotide sequence of a human cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in the deposit given the ATCC Deposit Number shown in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said sequence of at least 50 contiguous nucleotides is included in the nucleotide sequence of the complete open reading frame sequence encoded by said human cDNA clone.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 150 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 500 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is a method for detecting in a biological sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained

in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing a nucleotide sequence of at least one nucleic acid molecule in said sample with a sequence selected from said group and determining whether the sequence of said nucleic acid molecule in said sample is at least 95% identical to said selected sequence.

Also preferred is the above method wherein said step of comparing sequences comprises determining the extent of nucleic acid hybridization between nucleic acid molecules in said sample and a nucleic acid molecule comprising said sequence selected from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a nucleic acid molecule in said sample with said sequence selected from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

A further preferred embodiment is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting nucleic acid molecules in said sample, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for identifying the species, tissue or cell type of a biological sample can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject nucleic acid molecules, if any,



comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA  
5 Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for diagnosing a pathological condition can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least  
10 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a panel of at least two nucleotide sequences, wherein at least one sequence in said  
15 panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA  
20 clone in Table 1. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1.

25 Also preferred is a polypeptide, wherein said sequence of contiguous amino acids is included in the amino acid sequence of SEQ ID NO:Y in the range of positions beginning with the residue at about the position of the First Amino Acid of the Secreted Portion and ending with the residue at about the Last Amino Acid of the Open Reading Frame as set forth for SEQ ID NO:Y in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid  
5 sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the complete amino acid sequence of SEQ ID NO:Y.

10 Further preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

15 Also preferred is a polypeptide wherein said sequence of contiguous amino acids is included in the amino acid sequence of a secreted portion of the secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

20 Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

25 Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

30 Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence of the secreted portion of the protein

encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is an isolated antibody which binds specifically to a  
5 polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained  
10 in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method for detecting in a biological sample a polypeptide comprising an amino acid sequence which is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group  
15 consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing an amino acid sequence of at least  
20 one polypeptide molecule in said sample with a sequence selected from said group and determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 10 contiguous amino acids.

Also preferred is the above method wherein said step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence  
25 selected from said group comprises determining the extent of specific binding of polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in  
30 Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA

clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is the above method wherein said step of comparing sequences is performed by comparing the amino acid sequence determined from a polypeptide molecule in said sample with said sequence selected from said group.

Also preferred is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting polypeptide molecules in said sample, if any, comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample, which method comprises a step of detecting polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the above group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

In any of these methods, the step of detecting said polypeptide molecules includes using an antibody.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence encoding a polypeptide wherein said polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encoding a polypeptide has been optimized for expression of said polypeptide in a prokaryotic host.

Also preferred is an isolated nucleic acid molecule, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method of making a recombinant vector comprising inserting any of the above isolated nucleic acid molecule into a vector. Also preferred is the recombinant vector produced by this method. Also preferred is a method of making a recombinant host cell comprising introducing the vector into a host cell, as well as the recombinant host cell produced by this method.

Also preferred is a method of making an isolated polypeptide comprising culturing this recombinant host cell under conditions such that said polypeptide is expressed and recovering said polypeptide. Also preferred is this method of making an isolated polypeptide, wherein said recombinant host cell is a eukaryotic cell and said polypeptide is a secreted portion of a human secreted protein comprising an amino acid sequence selected from the group consisting of: an amino acid sequence of

SEQ ID NO:Y beginning with the residue at the position of the First Amino Acid of the Secreted Portion of SEQ ID NO:Y wherein Y is an integer set forth in Table 1 and said position of the First Amino Acid of the Secreted Portion of SEQ ID NO:Y is defined in Table 1; and an amino acid sequence of a secreted portion of a protein  
5 encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. The isolated polypeptide produced by this method is also preferred.

Also preferred is a method of treatment of an individual in need of an increased level of a secreted protein activity, which method comprises administering  
10 to such an individual a pharmaceutical composition comprising an amount of an isolated polypeptide, polynucleotide, or antibody of the claimed invention effective to increase the level of said protein activity in said individual.

The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel,  
15 horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate, and human. In specific embodiments, the host is a mouse, rabbit, goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

20

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

25

### Examples

#### Example 1: Isolation of a Selected cDNA Clone From the Deposited Sample

Each cDNA clone in a cited ATCC deposit is contained in a plasmid vector. Table 1 identifies the vectors used to construct the cDNA library from which each  
30 clone was isolated. In many cases, the vector used to construct the library is a phage vector from which a plasmid has been excised. The table immediately below

correlates the related plasmid for each phage vector used in constructing the cDNA library. For example, where a particular clone is identified in Table 1 as being isolated in the vector "Lambda Zap," the corresponding deposited clone is in "pBluescript."

|    |   |                                |
|----|---|--------------------------------|
| 5  | <u>Vector Used to Construct Library</u> | <u>Corresponding Deposited</u> |
|    | <u>Plasmid</u>                          |                                |
|    | Lambda Zap                              | pBluescript (pBS)              |
|    | Uni-Zap XR                              | pBluescript (pBS)              |
|    | Zap Express                             | pBK                            |
| 10 | lafmid BA                               | plafmid BA                     |
|    | pSport1                                 | pSport1                        |
|    | pCMVSPORT 2.0                           | pCMVSPORT 2.0                  |
|    | pCMVSPORT 3.0                           | pCMVSPORT 3.0                  |
|    | pCR <sup>®</sup> 2.1                    | pCR <sup>®</sup> 2.1           |

15 Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., Nucleic Acids Res. 16:7583-7600 (1988); Alting-Mees, M. A. and Short, J. M., Nucleic Acids Res. 17:9494 (1989)) and pBK (Alting-Mees, M. A. et al., Strategies 5:58-61 (1992)) are  
 20 commercially available from Stratagene Cloning Systems, Inc., 11011 N. Torrey Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Both can be transformed into E. coli strain XL-1 Blue, also available from Stratagene. pBS comes in 4 forms SK+, SK-, KS+ and KS-. The S and K refers to the orientation of the polylinker to the T7 and T3  
 25 primer sequences which flank the polylinker region ("S" is for SacI and "K" is for KpnI which are the first sites on each respective end of the linker). "+" or "-" refer to the orientation of the f1 origin of replication ("ori"), such that in one orientation, single stranded rescue initiated from the f1 ori generates sense strand DNA and in the other, antisense.

30 Vectors pSport1, pCMVSPORT 2.0 and pCMVSPORT 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors

contain an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, also available from Life Technologies. (See, for instance, Gruber, C. E., et al., *Focus* 15:59 (1993).) Vector lafmid BA (Bento Soares, Columbia University, NY) contains an ampicillin resistance gene and can be transformed into *E. coli* strain XL-1 Blue. Vector pCR<sup>®</sup>2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, available from Life Technologies. (See, for instance, Clark, J. M., *Nuc. Acids Res.* 16:9677-9686 (1988) and Mead, D. et al., *Bio/Technology* 9: (1991).) Preferably, a polynucleotide of the present invention does not comprise the phage vector sequences identified for the particular clone in Table 1, as well as the corresponding plasmid vector sequences designated above.

The deposited material in the sample assigned the ATCC Deposit Number cited in Table 1 for any given cDNA clone also may contain one or more additional plasmids, each comprising a cDNA clone different from that given clone. Thus, deposits sharing the same ATCC Deposit Number contain at least a plasmid for each cDNA clone identified in Table 1. Typically, each ATCC deposit sample cited in Table 1 comprises a mixture of approximately equal amounts (by weight) of about 50 plasmid DNAs, each containing a different cDNA clone; but such a deposit sample may include plasmids for more or less than 50 cDNA clones, up to about 500 cDNA clones.

Two approaches can be used to isolate a particular clone from the deposited sample of plasmid DNAs cited for that clone in Table 1. First, a plasmid is directly isolated by screening the clones using a polynucleotide probe corresponding to SEQ ID NO:X.

Particularly, a specific polynucleotide with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with <sup>32</sup>P-γ-ATP using T4 polynucleotide kinase and purified according to routine methods. (E.g., Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring, NY (1982).) The plasmid mixture is transformed into a suitable host, as indicated above (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as



those provided by the vector supplier or in related publications or patents cited above. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are screened using Nylon membranes according to routine methods for  
5 bacterial colony screening (e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

Alternatively, two primers of 17-20 nucleotides derived from both ends of the SEQ ID NO:X (i.e., within the region of SEQ ID NO:X bounded by the 5' NT and  
10 the 3' NT of the clone defined in Table 1) are synthesized and used to amplify the desired cDNA using the deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25 ul of reaction mixture with 0.5 ug of the above cDNA template. A convenient reaction mixture is 1.5-5 mM MgCl<sub>2</sub>, 0.01% (w/v) gelatin, 20 uM each of dATP, dCTP, dGTP, dTTP, 25  
15 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94 degree C for 1 min; annealing at 55 degree C for 1 min; elongation at 72 degree C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR  
20 product is verified to be the selected sequence by subcloning and sequencing the DNA product.

Several methods are available for the identification of the 5' or 3' non-coding portions of a gene which may not be present in the deposited clone. These methods include but are not limited to, filter probing, clone enrichment using specific probes,  
25 and protocols similar or identical to 5' and 3' "RACE" protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et al., *Nucleic Acids Res.* 21(7):1683-1684 (1993).)

Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a  
30 population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a

primer specific to a known sequence of the gene of interest is used to PCR amplify the 5' portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full length gene.

This above method starts with total RNA isolated from the desired source, although poly-A+ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the desired gene.

#### **Example 2: Isolation of Genomic Clones Corresponding to a Polynucleotide**

A human genomic PI library (Genomic Systems, Inc.) is screened by PCR using primers selected for the cDNA sequence corresponding to SEQ ID NO:X., according to the method described in Example 1. (See also, Sambrook.)

#### **Example 3: Tissue Distribution of Polypeptide**

Tissue distribution of mRNA expression of polynucleotides of the present invention is determined using protocols for Northern blot analysis, described by, among others, Sambrook et al. For example, a cDNA probe produced by the method described in Example 1 is labeled with P<sup>32</sup> using the rediprime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The

purified labeled probe is then used to examine various human tissues for mRNA expression.

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) (Clontech) are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70 degree C overnight, and the films developed according to standard procedures.

#### 10 **Example 4: Chromosomal Mapping of the Polynucleotides**

An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:X. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions : 30 seconds, 95 degree C; 1 minute, 56 degree C; 1 minute, 70 degree C. This cycle is repeated 32 times followed by one 5 minute cycle at 70 degree C. Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions is analyzed on either 8% polyacrylamide gels or 3.5 % agarose gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

#### **Example 5: Bacterial Expression of a Polypeptide**

A polynucleotide encoding a polypeptide of the present invention is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, such as BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp<sup>r</sup>), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a

ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

The pQE-9 vector is digested with BamHI and XbaI and the amplified  
fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at  
the bacterial RBS. The ligation mixture is then used to transform the E. coli strain  
5 M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which  
expresses the lacI repressor and also confers kanamycin resistance (Kan<sup>r</sup>).  
Transformants are identified by their ability to grow on LB plates and  
ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and  
10 confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid  
culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml).  
The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The  
cells are grown to an optical density 600 (O.D.<sup>600</sup>) of between 0.4 and 0.6. IPTG  
15 (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1  
mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to  
increased gene expression.

Cells are grown for an extra 3 to 4 hours. Cells are then harvested by  
centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic  
20 agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4 degree C. The cell debris  
is removed by centrifugation, and the supernatant containing the polypeptide is loaded  
onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from  
QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with  
high affinity and can be purified in a simple one-step procedure (for details see: The  
25 QIAexpressionist (1995) QIAGEN, Inc., *supra*).

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH  
8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then  
washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is  
eluted with 6 M guanidine-HCl, pH 5.

30 The purified protein is then renatured by dialyzing it against phosphate-  
buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl.

Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4 degree C or frozen at -80 degree C.

In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements operatively linked to a polynucleotide of the present invention, called pHE4a. (ATCC Accession Number 209645, deposited on February 25, 1998.) This vector contains: 1) a neomycinphosphotransferase gene as a selection marker, 2) an *E. coli* origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (*lacIq*). The origin of replication (*oriC*) is derived from pUC19 (LTI, Gaithersburg, MD). The promoter sequence and operator sequences are made synthetically.

DNA can be inserted into the pHEa by restricting the vector with NdeI and XbaI, BamHI, XhoI, or Asp718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is generated according to the PCR protocol described in Example 1, using PCR primers having restriction sites for NdeI (5' primer) and XbaI, BamHI, XhoI, or Asp718 (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

#### **Example 6: Purification of a Polypeptide from an Inclusion Body**

The following alternative method can be used to purify a polypeptide expressed in *E. coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10 degree C.

Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10 degree C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an  
5 appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

The cells are then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate  
10 is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the  
15 pellet is discarded and the polypeptide containing supernatant is incubated at 4 degree C overnight to allow further GuHCl extraction.

Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA  
20 by vigorous stirring. The refolded diluted protein solution is kept at 4 degree C without mixing for 12 hours prior to further purification steps.

To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 um membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The  
25 filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

30 Fractions containing the polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of

tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using  
5 a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant  $A_{280}$  monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant polypeptide should exhibit greater than 95% purity after the  
10 above refolding and purification steps. No major contaminant bands should be observed from Commassie blue stained 16% SDS-PAGE gel when 5 ug of purified protein is loaded. The purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

15

#### **Example 7: Cloning and Expression of a Polypeptide in a Baculovirus Expression System**

In this example, the plasmid shuttle vector pA2 is used to insert a polynucleotide into a baculovirus to express a polypeptide. This expression vector  
20 contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak  
25 *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

Many other baculovirus vectors can be used in place of the vector above, such  
30 as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription,

translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., *Virology* 170:31-39 (1989).

Specifically, the cDNA sequence contained in the deposited clone, including  
5 the AUG initiation codon and the naturally associated leader sequence identified in Table 1, is amplified using the PCR protocol described in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the pA2 vector does not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard methods described in  
10 Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment  
15 then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a  
20 commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.).

The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified  
25 by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

Five ug of a plasmid containing the polynucleotide is co-transfected with 1.0 ug of a commercially available linearized baculovirus DNA ("BaculoGold™  
30 baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., *Proc. Natl. Acad. Sci. USA* 84:7413-7417 (1987). One ug



of BaculoGold™ virus DNA and 5 ug of the plasmid are mixed in a sterile well of a microtiter plate containing 50 ul of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 ul Lipofectin plus 90 ul Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the  
5 transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27 degrees C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27 degrees C for four days.

10 After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell  
15 culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 ul of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded  
20 in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4 degree C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of  
25 infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 uCi of <sup>35</sup>S-methionine and 5 uCi <sup>35</sup>S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation.  
30 The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced protein.

**Example 8: Expression of a Polypeptide in Mammalian Cells**

5       The polypeptide of the present invention can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening  
10 sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

15       Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109), pCMVSPORT 2.0, and pCMVSPORT 3.0. Mammalian host cells that could be used include, human HeLa, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1,  
20 Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

      Alternatively, the polypeptide can be expressed in stable cell lines containing the polynucleotide integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification  
25 and isolation of the transfected cells.

      The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt, F. W., et al., J. Biol. Chem. 253:1357-1370 (1978); Hamlin, J.  
30 L. and Ma, C., Biochem. et Biophys. Acta, 1097:107-143 (1990); Page, M. J. and

Sydenham, M. A., *Biotechnology* 9:64-68 (1991).) Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., *Biochem J.* 227:277-279 (1991); Bebbington et al., *Bio/Technology* 10:169-175 (1992). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest  
5 resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession  
10 No.209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., *Molecular and Cellular Biology*, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., *Cell* 41:521-530 (1985).) Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors also contain the 3' intron, the  
15 polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

Specifically, the plasmid pC6, for example, is digested with appropriate restriction enzymes and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

20 A polynucleotide of the present invention is amplified according to the protocol outlined in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the vector does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

25 The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

30 The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then

transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction-enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five  $\mu$ g of the expression plasmid pC6 a pC4 is cotransfected with 0.5  $\mu$ g of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1  $\mu$ M, 2  $\mu$ M, 5  $\mu$ M, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200  $\mu$ M. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

#### 20 Example 9: Protein Fusions

The polypeptides of the present invention are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of the present polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See Example 5; see also EP A 394,827; Traunecker, et al., Nature 331:84-86 (1988).) Similarly, fusion to IgG-1, IgG-3, and albumin increases the halflife time in vivo. Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused

protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 5.

5 Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

10 For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and a polynucleotide of the present invention, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

15 If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

20 Human IgG Fc region:

GGGATCCGGAGCCCAAATCTTCTGACAAACTCACACATGCCCACCGTGC  
CCAGCACCTGAATTCGAGGGTGCACCGTCAGTCTTCCTCTTCCCCCAAAA  
CCCAAGGACACCCTCATGATCTCCCGGACTCCTGAGGTCACATGCGTGGT  
GGTGGACGTAAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGG  
25 ACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTA  
CAACAGCACGTACCGTGTGGTCAGCGTCCTACCGTCCTGCACCAGGACT  
GGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCA  
ACCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC  
CACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAG  
30 GTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCGCCGT  
GGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCT

CCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTG  
GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCA  
TGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGG  
GTAAATGAGTGCGACGGCCGCGACTCTAGAGGAT (SEQ ID NO:1)

5

**Example 10: Production of an Antibody from a Polypeptide**

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing a polypeptide of the present invention is administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of the secreted protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology. (Köhler et al., Nature 256:495 (1975); Köhler et al., Eur. J. Immunol. 6:511 (1976); Köhler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981).) In general, such procedures involve immunizing an animal (preferably a mouse) with polypeptide or, more preferably, with a secreted polypeptide-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56 degrees C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 ug/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981).) The hybridoma

cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide.

Alternatively, additional antibodies capable of binding to the polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the protein-specific antibody can be blocked by the polypeptide. Such antibodies comprise anti-idiotypic antibodies to the protein-specific antibody and can be used to immunize an animal to induce formation of further protein-specific antibodies.

It will be appreciated that Fab and F(ab')<sub>2</sub> and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). Alternatively, secreted protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

For in vivo use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

**Example 11: Production Of Secreted Protein For High-Throughput Screening****Assays**

The following protocol produces a supernatant containing a polypeptide to be tested. This supernatant can then be used in the Screening Assays described in

5 Examples 13-20.

First, dilute Poly-D-Lysine (644 587 Boehringer-Mannheim) stock solution (1mg/ml in PBS) 1:20 in PBS (w/o calcium or magnesium 17-516F Biowhittaker) for a working solution of 50ug/ml. Add 200 ul of this solution to each well (24 well plates) and incubate at RT for 20 minutes. Be sure to distribute the solution over each  
10 well (note: a 12-channel pipetter may be used with tips on every other channel). Aspirate off the Poly-D-Lysine solution and rinse with 1ml PBS (Phosphate Buffered Saline). The PBS should remain in the well until just prior to plating the cells and plates may be poly-lysine coated in advance for up to two weeks.

Plate 293T cells (do not carry cells past P+20) at  $2 \times 10^5$  cells/well in .5ml  
15 DMEM(Dulbecco's Modified Eagle Medium)(with 4.5 G/L glucose and L-glutamine (12-604F Biowhittaker))/10% heat inactivated FBS(14-503F Biowhittaker)/1x Penstrep(17-602E Biowhittaker). Let the cells grow overnight.

The next day, mix together in a sterile solution basin: 300 ul Lipofectamine (18324-012 Gibco/BRL) and 5ml Optimem I (31985070 Gibco/BRL)/96-well plate.  
20 With a small volume multi-channel pipetter, aliquot approximately 2ug of an expression vector containing a polynucleotide insert, produced by the methods described in Examples 8 or 9, into an appropriately labeled 96-well round bottom plate. With a multi-channel pipetter, add 50ul of the Lipofectamine/Optimem I mixture to each well. Pipette up and down gently to mix. Incubate at RT 15-45  
25 minutes. After about 20 minutes, use a multi-channel pipetter to add 150ul Optimem I to each well. As a control, one plate of vector DNA lacking an insert should be transfected with each set of transfections.

Preferably, the transfection should be performed by tag-teaming the following tasks. By tag-teaming, hands on time is cut in half, and the cells do not spend too  
30 much time on PBS. First, person A aspirates off the media from four 24-well plates of cells, and then person B rinses each well with .5-1ml PBS. Person A then aspirates



off PBS rinse, and person B, using a 12-channel pipetter with tips on every other channel, adds the 200ul of DNA/Lipofectamine/Optimem I complex to the odd wells first, then to the even wells, to each row on the 24-well plates. Incubate at 37 degrees C for 6 hours.

- 5 While cells are incubating, prepare appropriate media, either 1%BSA in DMEM with 1x penstrep, or CHO-5 media (116.6 mg/L of  $\text{CaCl}_2$  (anhyd); 0.00130 mg/L  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ; 0.050 mg/L of  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ; 0.417 mg/L of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ; 311.80 mg/L of KCl; 28.64 mg/L of  $\text{MgCl}_2$ ; 48.84 mg/L of  $\text{MgSO}_4$ ; 6995.50 mg/L of NaCl; 2400.0 mg/L of  $\text{NaHCO}_3$ ; 62.50 mg/L of  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ; 71.02 mg/L of
- 10  $\text{Na}_2\text{HPO}_4$ ; .4320 mg/L of  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ; .002 mg/L of Arachidonic Acid ; 1.022 mg/L of Cholesterol; .070 mg/L of DL-alpha-Tocopherol-Acetate; 0.0520 mg/L of Linoleic Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of Oleic Acid; 0.010 mg/L of Palmitic Acid; 0.010 mg/L of Palmitic Acid; 100 mg/L of Pluronic F-68; 0.010 mg/L of Stearic Acid; 2.20 mg/L of Tween 80; 4551 mg/L of D-
- 15 Glucose; 130.85 mg/ml of L- Alanine; 147.50 mg/ml of L-Arginine-HCL; 7.50 mg/ml of L-Asparagine- $\text{H}_2\text{O}$ ; 6.65 mg/ml of L-Aspartic Acid; 29.56 mg/ml of L-Cystine-2HCL- $\text{H}_2\text{O}$ ; 31.29 mg/ml of L-Cystine-2HCL; 7.35 mg/ml of L-Glutamic Acid; 365.0 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52.48 mg/ml of L-Histidine-HCL- $\text{H}_2\text{O}$ ; 106.97 mg/ml of L-Isoleucine; 111.45 mg/ml of L-Leucine; 163.75 mg/ml of L-
- 20 Lysine HCL; 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalanine; 40.0 mg/ml of L-Proline; 26.25 mg/ml of L-Serine; 101.05 mg/ml of L-Threonine; 19.22 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tyrosine-2Na-2 $\text{H}_2\text{O}$ ; 99.65 mg/ml of L-Valine; 0.0035 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate; 11.78 mg/L of Choline Chloride; 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol; 3.02 mg/L of
- 25 Niacinamide; 3.00 mg/L of Pyridoxal HCL; 0.031 mg/L of Pyridoxine HCL; 0.319 mg/L of Riboflavin; 3.17 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; and 0.680 mg/L of Vitamin B<sub>12</sub>; 25 mM of HEPES Buffer; 2.39 mg/L of Na Hypoxanthine; 0.105 mg/L of Lipoic Acid; 0.081 mg/L of Sodium Putrescine-2HCL; 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 20uM of
- 30 Ethanolamine; 0.122 mg/L of Ferric Citrate; 41.70 mg/L of Methyl-B-Cyclodextrin complexed with Linoleic Acid; 33.33 mg/L of Methyl-B-Cyclodextrin complexed

with Oleic Acid; and 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal) with 2mm glutamine and 1x penstrep. (BSA (81-068-3 Bayer) 100gm dissolved in 1L DMEM for a 10% BSA stock solution). Filter the media and collect 50 ul for endotoxin assay in 15ml polystyrene conical.

5        The transfection reaction is terminated, preferably by tag-teaming, at the end of the incubation period. Person A aspirates off the transfection media, while person B adds 1.5ml appropriate media to each well. Incubate at 37 degrees C for 45 or 72 hours depending on the media used: 1%BSA for 45 hours or CHO-5 for 72 hours.

10        On day four, using a 300ul multichannel pipetter, aliquot 600ul in one 1ml deep well plate and the remaining supernatant into a 2ml deep well. The supernatants from each well can then be used in the assays described in Examples 13-20.

15        It is specifically understood that when activity is obtained in any of the assays described below using a supernatant, the activity originates from either the polypeptide directly (e.g., as a secreted protein) or by the polypeptide inducing expression of other proteins, which are then secreted into the supernatant. Thus, the invention further provides a method of identifying the protein in the supernatant characterized by an activity in a particular assay.

#### **Example 12: Construction of GAS Reporter Construct**

20        One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the Jaks-STATs pathway bind to gamma activation site "GAS" elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a protein to these elements alter the expression of the associated gene.

25        GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or "STATs." There are six members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in many cell types though it has been found in T helper class I, cells after treatment with  
30 IL-12. Stat5 was originally called mammary growth factor, but has been found at

higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many cytokines.

The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive in resting cells.

The Jaks are activated by a wide range of receptors summarized in the Table below. (Adapted from review by Schidler and Darnell, Ann. Rev. Biochem. 64:621-51 (1995).) A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; and (b) Class 2 includes IFN- $\alpha$ , IFN- $\gamma$ , and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proximal region encoding Trp-Ser-Xxx-Trp-Ser (SEQ ID NO:2)).

Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn activate STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway.

Therefore, activation of the Jaks-STATs pathway, reflected by the binding of the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines are known to activate the Jaks-STATs pathway. (See Table below.) Thus, by using GAS elements linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.

|                                  | <u>Ligand</u>        | <u>JAKs</u> |             |             |             | <u>STATS</u> | <u>GAS(elements) or ISRE</u> |
|----------------------------------|----------------------|-------------|-------------|-------------|-------------|--------------|------------------------------|
|                                  |                      | <u>tyk2</u> | <u>Jak1</u> | <u>Jak2</u> | <u>Jak3</u> |              |                              |
| <u>IFN family</u>                |                      |             |             |             |             |              |                              |
| 5                                | IFN-a/B              | +           | +           | -           | -           | 1,2,3        | ISRE<br>GAS (IRF1>Lys6>IFP)  |
|                                  | IFN-g                |             | +           | +           | -           | 1            |                              |
|                                  | Il-10                | +           | ?           | ?           | -           | 1,3          |                              |
| <u>gp130 family</u>              |                      |             |             |             |             |              |                              |
| 10                               | IL-6 (Pleiotrophic)  | +           | +           | +           | ?           | 1,3          | GAS (IRF1>Lys6>IFP)          |
|                                  | Il-11(Pleiotrophic)  | ?           | +           | ?           | ?           | 1,3          |                              |
|                                  | OnM(Pleiotrophic)    | ?           | +           | +           | ?           | 1,3          |                              |
|                                  | LIF(Pleiotrophic)    | ?           | +           | +           | ?           | 1,3          |                              |
|                                  | CNTF(Pleiotrophic)   | -/+         | +           | +           | ?           | 1,3          |                              |
| 15                               | G-CSF(Pleiotrophic)  | ?           | +           | ?           | ?           | 1,3          |                              |
|                                  | IL-12(Pleiotrophic)  | +           | -           | +           | +           | 1,3          |                              |
| <u>g-C family</u>                |                      |             |             |             |             |              |                              |
| 20                               | IL-2 (lymphocytes)   | -           | +           | -           | +           | 1,3,5        | GAS                          |
|                                  | IL-4 (lymph/myeloid) | -           | +           | -           | +           | 6            | GAS (IRF1 = IFP >>Ly6)(IgH)  |
|                                  | IL-7 (lymphocytes)   | -           | +           | -           | +           | 5            | GAS                          |
|                                  | IL-9 (lymphocytes)   | -           | +           | -           | +           | 5            | GAS                          |
|                                  | IL-13 (lymphocyte)   | -           | +           | ?           | ?           | 6            | GAS                          |
| 25                               | IL-15                | ?           | +           | ?           | +           | 5            | GAS                          |
| <u>gp140 family</u>              |                      |             |             |             |             |              |                              |
|                                  | IL-3 (myeloid)       | -           | -           | +           | -           | 5            | GAS (IRF1>IFP>>Ly6)          |
|                                  | IL-5 (myeloid)       | -           | -           | +           | -           | 5            |                              |
| 30                               | GM-CSF (myeloid)     | -           | -           | +           | -           | 5            |                              |
| <u>Growth hormone family</u>     |                      |             |             |             |             |              |                              |
|                                  | GH                   | ?           | -           | +           | -           | 5            | GAS(B-CAS>IRF1=IFP>>Ly6)     |
|                                  | PRL                  | ?           | +/-         | +           | -           | 1,3,5        |                              |
| 35                               | EPO                  | ?           | -           | +           | -           | 5            |                              |
| <u>Receptor Tyrosine Kinases</u> |                      |             |             |             |             |              |                              |
|                                  | EGF                  | ?           | +           | +           | -           | 1,3          | GAS (IRF1)                   |
|                                  | PDGF                 | ?           | +           | +           | -           | 1,3          |                              |
| 40                               | CSF-1                | ?           | +           | +           | -           | 1,3          | GAS (not IRF1)               |

GAS (IRF1&gt;IFP&gt;&gt;Ly6)

GAS (IRF1)

GAS (not IRF1)

To construct a synthetic GAS containing promoter element, which is used in the Biological Assays described in Examples 13-14, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman et al., Immunity 1:457-468 (1994).), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18bp of sequence complementary to the SV40 early promoter sequence and is flanked with an XhoI site. The sequence of the 5' primer is:

5':GCGCCTCGAGATTTCCTCCGAAATCTAGATTTCCTCCGAAATGATTTCCTCCGAAATGATTTCCTCCGAAATATCTGCCATCTCAATTAG:3' (SEQ ID NO:3)

The downstream primer is complementary to the SV40 promoter and is flanked with a Hind III site: 5':GCGGCAAGCTTTTGTCAAAGCCTAGGC:3' (SEQ ID NO:4)

PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI/Hind III and subcloned into BLSK2-. (Stratagene.) Sequencing with forward and reverse primers confirms that the insert contains the following sequence:

5':CTCGAGATTTCCTCCGAAATCTAGATTTCCTCCGAAATGATTTCCTCCGAAATGATTTCCTCCGAAATATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAGCTT:3' (SEQ ID NO:5)

With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP." Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol

acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using HindIII and XhoI, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Thus, in order to generate mammalian stable cell lines expressing the GAS-SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector using SalI and NotI, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding as described in Examples 13-14.

Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NFK-B and EGR promoter sequences are described in Examples 15 and 16. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, IL-2/NFAT, or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

### **Example 13: High-Throughput Screening Assay for T-cell Activity.**

The following protocol is used to assess T-cell activity by identifying factors, and determining whether supernate containing a polypeptide of the invention proliferates and/or differentiates T-cells. T-cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway.

The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152), although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.

5 Jurkat T-cells are lymphoblastic CD4+ Th1 helper cells. In order to generate stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DMRIE-C (Life Technologies)(transfection procedure described below). The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to 1 mg/ml gentamicin selected. Resistant colonies are expanded and then tested for their response to increasing  
10 concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells containing 200 ul of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in  
15 RPMI + 10% serum with 1%Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life Technologies) with 10 ug of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 ul of DMRIE-C and incubate at room temperature for 15-45 mins.

During the incubation period, count cell concentration, spin down the required number of cells ( $10^7$  per transfection), and resuspend in OPTI-MEM to a final  
20 concentration of  $10^7$  cells/ml. Then add 1ml of  $1 \times 10^7$  cells in OPTI-MEM to T25 flask and incubate at 37 degrees C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Gentamicin, and 1% Pen-Strep. These cells are treated with  
25 supernatants containing polypeptides of the invention and/or induced polypeptides of the invention as produced by the protocol described in Example 11.

On the day of treatment with the supernatant, the cells should be washed and resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of supernatants being  
30 screened. For one 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.

Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200 ul of cells into each well (therefore adding 100, 000 cells per well).

After all the plates have been seeded, 50 ul of the supernatants are transferred directly from the 96 well plate containing the supernatants into each well using a 12 channel pipette. In addition, a dose of exogenous interferon gamma (0.1, 1.0, 10 ng) is added to wells H9, H10, and H11 to serve as additional positive controls for the assay.

The 96 well dishes containing Jurkat cells treated with supernatants are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35 ul samples from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophene covers) and stored at -20 degrees C until SEAP assays are performed according to Example 17. The plates containing the remaining treated cells are placed at 4 degrees C and serve as a source of material for repeating the assay on a specific well if desired.

As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

The above protocol may be used in the generation of both transient, as well as, stable transfected cells, which would be apparent to those of skill in the art.

#### **Example 14: High-Throughput Screening Assay Identifying Myeloid Activity**

The following protocol is used to assess myeloid activity by determining whether polypeptides of the invention proliferates and/or differentiates myeloid cells.

Myeloid cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

To transiently transfect U937 cells with the GAS/SEAP/Neo construct produced in Example 12, a DEAE-Dextran method (Kharbanda et. al., 1994, Cell Growth & Differentiation, 5:259-265) is used. First, harvest  $2 \times 10^7$  U937 cells and



wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing  
5 0.5 mg/ml DEAE-Dextran, 8 ug GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM KCl, 375 uM Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O, 1 mM MgCl<sub>2</sub>, and 675 uM CaCl<sub>2</sub>. Incubate at 37 degrees C for 45 min.

Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37 degrees C for 36 hr.

10 The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400 ug/ml G418 for couple of passages.

These cells are tested by harvesting  $1 \times 10^8$  cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 ml above described  
15 growth medium, with a final density of  $5 \times 10^5$  cells/ml. Plate 200 ul cells per well in the 96-well plate (or  $1 \times 10^5$  cells/well).

Add 50 ul of the supernatant prepared by the protocol described in Example  
11. Incubate at 37 degrees C for 48 to 72 hr. As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate U937 cells. Over 30 fold  
20 induction is typically observed in the positive control wells. SEAP assay the supernatant according to the protocol described in Example 17.

#### **Example 15: High-Throughput Screening Assay Identifying Neuronal Activity.**

When cells undergo differentiation and proliferation, a group of genes are  
25 activated through many different signal transduction pathways. One of these genes, EGR1 (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGR1 is responsible for such induction. Using the EGR1 promoter linked to reporter molecules, activation of cells can be assessed.

Particularly, the following protocol is used to assess neuronal activity in PC12  
30 cell lines. PC12 cells (rat phenochromocytoma cells) are known to proliferate and/or differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl

phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor). The EGR1 gene expression is activated during this treatment. Thus, by stably transfecting PC12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells can be assessed.

5       The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-1 promoter sequence (-633 to +1)(Sakamoto K et al., Oncogene 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers:

5' GCGCTCGAGGGATGACAGCGATAGAACCCCGG -3' (SEQ ID NO:6)

10       5' GCGAAGCTTCGCGACTCCCCGGATCCGCCTC-3' (SEQ ID NO:7)

Using the GAS:SEAP/Neo vector produced in Example 12, EGR1 amplified product can then be inserted into this vector. Linearize the GAS:SEAP/Neo vector using restriction enzymes XhoI/HindIII, removing the GAS/SV40 stuffer. Restrict the EGR1 amplified product with these same enzymes. Ligate the vector and the EGR1  
15       promoter.

To prepare 96 well-plates for cell culture, two mls of a coating solution (1:30 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one 10 cm plate or 50 ml per well of the 96-well plate, and allowed to air dry for 2 hr.

20       PC12 cells are routinely grown in RPMI-1640 medium (Bio Whittaker) containing 10% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 ug/ml streptomycin on a precoated 10 cm tissue culture dish. One to four split is done every three to four days. Cells are removed from the plates by scraping and  
25       resuspended with pipetting up and down for more than 15 times.

Transfect the EGR/SEAP/Neo construct into PC12 using the Lipofectamine protocol described in Example 11. EGR-SEAP/PC12 stable cells are obtained by growing the cells in 300 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 300 ug/ml G418  
30       for couple of passages.

To assay for neuronal activity, a 10 cm plate with cells around 70 to 80% confluent is screened by removing the old medium. Wash the cells once with PBS (Phosphate buffered saline). Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

5        The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count the cell number and add more low serum medium to reach final cell density as  $5 \times 10^5$  cells/ml.

10        Add 200 ul of the cell suspension to each well of 96-well plate (equivalent to  $1 \times 10^5$  cells/well). Add 50 ul supernatant produced by Example 11, 37°C for 48 to 72 hr. As a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/ul of Neuronal Growth Factor (NGF). Over fifty-fold induction of SEAP is typically seen in the positive control wells. SEAP assay the supernatant according to Example 17.

15

#### **Example 16: High-Throughput Screening Assay for T-cell Activity**

NF-KB (Nuclear Factor KB) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF-KB regulates the expression of genes involved in immune cell activation, control of apoptosis (NF- KB appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

20        In non-stimulated conditions, NF- KB is retained in the cytoplasm with I-KB (Inhibitor KB). However, upon stimulation, I- KB is phosphorylated and degraded, causing NF- KB to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF- KB include IL-2, IL-6, GM-CSF, ICAM-1 and class 1 MHC.

25        Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF-KB promoter element are used to screen the supernatants produced in Example 11. Activators or inhibitors of NF-KB would be useful in

30

treating diseases. For example, inhibitors of NF-KB could be used to treat those diseases related to the acute or chronic activation of NF-KB, such as rheumatoid arthritis.

To construct a vector containing the NF-KB promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF-KB binding site (GGGGACTTTCCC) (SEQ ID NO:8), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an XhoI site:

5':GCGGCCTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGAC  
TTTCCATCCTGCCATCTCAATTAG:3' (SEQ ID NO:9)

The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a Hind III site:

5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:4)

PCR amplification is performed using the SV40 promoter template present in the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI and Hind III and subcloned into BLSK2-. (Stratagene)

Sequencing with the T7 and T3 primers confirms the insert contains the following sequence:

5':CTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGGACTTTCC  
ATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCC  
ATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGA  
CTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTA  
TTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAA  
GCTT:3' (SEQ ID NO:10)

Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this NF-KB/SV40 fragment using XhoI and HindIII. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

In order to generate stable mammalian cell lines, the NF-KB/SV40/SEAP cassette is removed from the above NF-KB/SEAP vector using restriction enzymes SalI and NotI, and inserted into a vector containing neomycin resistance. Particularly, the NF-KB/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with SalI and NotI.

Once NF-KB/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 13. Similarly, the method for assaying supernatants with these stable Jurkat T-cells is also described in Example 13. As a positive control, exogenous TNF alpha (0.1, 1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

#### **Example 17: Assay for SEAP Activity**

As a reporter molecule for the assays described in Examples 13-16, SEAP activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

Prime a dispenser with the 2.5x Dilution Buffer and dispense 15 ul of 2.5x dilution buffer into Optiplates containing 35 ul of a supernatant. Seal the plates with a plastic sealer and incubate at 65 degree C for 30 min. Separate the Optiplates to avoid uneven heating.

Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50 ml Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the table below). Add 50 ul Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on luminometer, one should treat 5 plates at each time and start the second set 10 minutes later.

Read the relative light unit in the luminometer. Set H12 as blank, and print the results. An increase in chemiluminescence indicates reporter activity.

#### **Reaction Buffer Formulation:**

| # of plates | Rxn buffer diluent (ml) | CSPD (ml) |
|-------------|-------------------------|-----------|
| 10          | 60                      | 3         |
| 11          | 65                      | 3.25      |
| 12          | 70                      | 3.5       |
| 13          | 75                      | 3.75      |
| 14          | 80                      | 4         |
| 15          | 85                      | 4.25      |
| 16          | 90                      | 4.5       |
| 17          | 95                      | 4.75      |
| 18          | 100                     | 5         |
| 19          | 105                     | 5.25      |
| 20          | 110                     | 5.5       |
| 21          | 115                     | 5.75      |
| 22          | 120                     | 6         |
| 23          | 125                     | 6.25      |
| 24          | 130                     | 6.5       |
| 25          | 135                     | 6.75      |
| 26          | 140                     | 7         |
| 27          | 145                     | 7.25      |
| 28          | 150                     | 7.5       |
| 29          | 155                     | 7.75      |
| 30          | 160                     | 8         |
| 31          | 165                     | 8.25      |
| 32          | 170                     | 8.5       |
| 33          | 175                     | 8.75      |
| 34          | 180                     | 9         |
| 35          | 185                     | 9.25      |
| 36          | 190                     | 9.5       |
| 37          | 195                     | 9.75      |
| 38          | 200                     | 10        |
| 39          | 205                     | 10.25     |
| 40          | 210                     | 10.5      |
| 41          | 215                     | 10.75     |
| 42          | 220                     | 11        |
| 43          | 225                     | 11.25     |
| 44          | 230                     | 11.5      |
| 45          | 235                     | 11.75     |
| 46          | 240                     | 12        |
| 47          | 245                     | 12.25     |
| 48          | 250                     | 12.5      |
| 49          | 255                     | 12.75     |
| 50          | 260                     | 13        |

**Example 18: High-Throughput Screening Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability**

5 Binding of a ligand to a receptor is known to alter intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify supernatants which bind to receptors of a particular cell. Although the following protocol

describes an assay for calcium, this protocol can easily be modified to detect changes in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a fluorescent probe.

5 The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-4 (Molecular Probes, Inc.; catalog no. F-14202), used here.

10 For adherent cells, seed the cells at 10,000 -20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in a CO<sub>2</sub> incubator for 20 hours. The adherent cells are washed two times in Biotek washer with 200 ul of HBSS (Hank's Balanced Salt Solution) leaving 100 ul of buffer after the final wash.

15 A stock solution of 1 mg/ml fluo-4 is made in 10% pluronic acid DMSO. To load the cells with fluo-4, 50 ul of 12 ug/ml fluo-4 is added to each well. The plate is incubated at 37 degrees C in a CO<sub>2</sub> incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 ul of buffer.

20 For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to 2-5x10<sup>6</sup> cells/ml with HBSS in a 50-ml conical tube. 4 ul of 1 mg/ml fluo-4 solution in 10% pluronic acid DMSO is added to each ml of cell suspension. The tube is then placed in a 37 degrees C water bath for 30-60 min. The cells are washed twice with HBSS, resuspended to 1x10<sup>6</sup> cells/ml, and dispensed into a microplate, 100 ul/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is then washed once in Denley CellWash with 200 ul, followed by an aspiration step to 100 ul final volume.

25 For a non-cell based assay, each well contains a fluorescent molecule, such as fluo-4. The supernatant is added to the well, and a change in fluorescence is detected.

30 To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm; and (6) Sample addition is 50 ul. Increased emission at 530 nm indicates an

extracellular signaling event which has resulted in an increase in the intracellular  $\text{Ca}^{++}$  concentration.

**Example 19: High-Throughput Screening Assay Identifying Tyrosine Kinase**

**5 Activity**

The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase (RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies.

- 10 In addition there are a large family of RPTKs for which the corresponding ligand is unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

- 15 Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., src, yes, lck, lyn, fyn) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

- 20 Because of the wide range of known factors capable of stimulating tyrosine kinase activity, the identification of novel human secreted proteins capable of activating tyrosine kinase signal transduction pathways are of interest. Therefore, the following protocol is designed to identify those novel human secreted proteins capable of activating the tyrosine kinase signal transduction pathways.

- 25 Seed target cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or  
30 polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or 10% Matrigel purchased from Becton Dickinson (Bedford, MA), or



calf serum, rinsed with PBS and stored at 4 degree C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamarBlue as described by the manufacturer Alamar Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford, MA) are used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20 minutes treatment with EGF (60ng/ml) or 50 ul of the supernatant produced in Example 11, the medium was removed and 100 ml of extraction buffer ((20 mM HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 2 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> and a cocktail of protease inhibitors (# 1836170) obtained from Boehringer Mannheim (Indianapolis, IN) is added to each well and the plate is shaken on a rotating shaker for 5 minutes at 4 degrees C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 mm membrane bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation, the content of each well, after detergent solubilization for 5 minutes, is removed and centrifuged for 15 minutes at 4 degrees C at 16,000 x g.

Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

Generally, the tyrosine kinase activity of a supernatant is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a biotinylated peptide). Biotinylated peptides that can be used for this purpose include PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

The tyrosine kinase reaction is set up by adding the following components in order. First, add 10ul of 5uM Biotinylated Peptide, then 10ul ATP/Mg<sub>2+</sub> (5mM ATP/50mM MgCl<sub>2</sub>), then 10ul of 5x Assay Buffer (40mM imidazole hydrochloride, pH7.3, 40 mM beta-glycerophosphate, 1mM EGTA, 100mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 0.5 mg/ml BSA), then 5ul of Sodium Vanadate(1mM), and then 5ul of water. Mix the components gently and preincubate the reaction mix at 30 degrees C for 2 min. Initial the reaction by adding 10ul of the control enzyme or the filtered supernatant.

The tyrosine kinase assay reaction is then terminated by adding 10 ul of 120mM EDTA and place the reactions on ice.

10 Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37 degrees C for 20 min. This allows the streptavidin coated 96 well plate to associate with the biotinylated peptide. Wash the MTP module with 300ul/well of PBS four times. Next add 75 ul of anti-phosphotyrosine antibody conjugated to horse radish  
15 peroxidase(anti-P-Tyr-POD(0.5u/ml)) to each well and incubate at 37 degrees C for one hour. Wash the well as above.

Next add 100ul of peroxidase substrate solution (Boehringer Mannheim) and incubate at room temperature for at least 5 mins (up to 30 min). Measure the absorbance of the sample at 405 nm by using ELISA reader. The level of bound  
20 peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

#### **Example 20: High-Throughput Screening Assay Identifying Phosphorylation Activity**

25 As a potential alternative and/or compliment to the assay of protein tyrosine kinase activity described in Example 19, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other  
30 molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase,

Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by substituting these molecules for Erk-1 or Erk-2 in the following assay.

Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0.1ml of protein G (1ug/ml) for 2 hr at room temp, (RT). The plates are then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100ng/well) against Erk-1 and Erk-2 (1 hr at RT) (Santa Cruz Biotechnology). (To detect other molecules, this step can easily be modified by substituting a monoclonal antibody detecting any of the above described molecules.) After 3-5 rinses with PBS, the plates are stored at 4 degrees C until use.

A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or 50 ul of the supernatants obtained in Example 11 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1ug/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation.

#### **Example 21: Method of Determining Alterations in a Gene Corresponding to a Polynucleotide**

RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is isolated. cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA

is then used as a template for PCR, employing primers surrounding regions of interest in SEQ ID NO:X. Suggested PCR conditions consist of 35 cycles at 95 degrees C for 30 seconds; 60-120 seconds at 52-58 degrees C; and 60-120 seconds at 70 degrees C, using buffer solutions described in Sidransky et al., Science 252:706 (1991).

5 PCR products are then sequenced using primers labeled at their 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre Technologies). The intron-exon borders of selected exons is also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations is then cloned and sequenced to validate the results of the direct  
10 sequencing.

PCR products is cloned into T-tailed vectors as described in Holton et al., Nucleic Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United States Biochemical). Affected individuals are identified by mutations not present in unaffected individuals.

15 Genomic rearrangements are also observed as a method of determining alterations in a gene corresponding to a polynucleotide. Genomic clones isolated according to Example 2 are nick-translated with digoxigenin deoxy-uridine 5'-triphosphate (Boehringer Mannheim), and FISH performed as described in Johnson et al., Methods Cell Biol. 35:73-99 (1991). Hybridization with the labeled probe is  
20 carried out using a vast excess of human cot-1 DNA for specific hybridization to the corresponding genomic locus.

Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology,  
25 Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson et al., Genet. Anal. Tech. Appl., 8:75 (1991).) Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of  
30 the genomic region hybridized by the probe are identified as insertions, deletions, and

translocations. These alterations are used as a diagnostic marker for an associated disease.

**Example 22: Method of Detecting Abnormal Levels of a Polypeptide in a Biological Sample**

A polypeptide of the present invention can be detected in a biological sample, and if an increased or decreased level of the polypeptide is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described in Example 10. The wells are blocked so that non-specific binding of the polypeptide to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample containing the polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbounded polypeptide.

Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

Add 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale).

Interpolate the concentration of the polypeptide in the sample using the standard curve.

**Example 23: Formulation**

The invention also provides methods of treatment and/or prevention of  
5 diseases or disorders (such as, for example, any one or more of the diseases or  
disorders disclosed herein) by administration to a subject of an effective amount of a  
Therapeutic. By therapeutic is meant a polynucleotides or polypeptides of the  
invention (including fragments and variants), agonists or antagonists thereof, and/or  
antibodies thereto, in combination with a pharmaceutically acceptable carrier type  
10 (e.g., a sterile carrier).

The Therapeutic will be formulated and dosed in a fashion consistent with  
good medical practice, taking into account the clinical condition of the individual  
patient (especially the side effects of treatment with the Therapeutic alone), the site of  
delivery, the method of administration, the scheduling of administration, and other  
15 factors known to practitioners. The "effective amount" for purposes herein is thus  
determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of the  
Therapeutic administered parenterally per dose will be in the range of about  
1 ug/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this  
20 will be subject to therapeutic discretion. More preferably, this dose is at least 0.01  
mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for  
the hormone. If given continuously, the Therapeutic is typically administered at a  
dose rate of about 1 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per  
day or by continuous subcutaneous infusions, for example, using a mini-pump. An  
25 intravenous bag solution may also be employed. The length of treatment needed to  
observe changes and the interval following treatment for responses to occur appears  
to vary depending on the desired effect.

Therapeutics can be are administered orally, rectally, parenterally,  
intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments,  
30 gels, drops or transdermal patch), buccally, or as an oral or nasal spray.  
"Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid

filler, diluent, encapsulating material or formulation auxiliary of any. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

5       Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics are administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier"

10       refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

15       Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

20       Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., Biopolymers 22:547-556 (1983)), poly (2-hydroxyethyl methacrylate) (Langer et al., J. Biomed. Mater. Res. 15:167-277 (1981), and Langer, Chem. Tech. 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., Id.) or poly-D-(-)-3-hydroxybutyric

25       acid (EP 133,988).

30       Sustained-release Therapeutics also include liposomally entrapped Therapeutics of the invention (see generally, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317-327 and 353-365 (1989)). Liposomes containing the Therapeutic are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang

et al., Proc. Natl. Acad. Sci.(USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than  
5 about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Therapeutic.

In yet an additional embodiment, the Therapeutics of the invention are delivered by way of a pump (*see* Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J.  
10 Med. 321:574 (1989)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

For parenteral administration, in one embodiment, the Therapeutic is formulated generally by mixing it at the desired degree of purity, in a unit dosage  
15 injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to the Therapeutic.

20 Generally, the formulations are prepared by contacting the Therapeutic uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's  
25 solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as  
30 phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten



residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The Therapeutic is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

Any pharmaceutical used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutics generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Therapeutics ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous Therapeutic solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized Therapeutic using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the Therapeutics of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the Therapeutics may be employed in conjunction with other therapeutic compounds.

The Therapeutics of the invention may be administered alone or in combination with adjuvants. Adjuvants that may be administered with the

Therapeutics of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG, and MPL. In a specific embodiment, Therapeutics of the invention are administered in combination with alum. In another specific embodiment, Therapeutics of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the Therapeutics of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diphtheria, hepatitis A, hepatitis B, haemophilus influenzae B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

The Therapeutics of the invention may be administered alone or in combination with other therapeutic agents. Therapeutic agents that may be administered in combination with the Therapeutics of the invention, include but not limited to, other members of the TNF family, chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, cytokines and/or growth factors. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination"

further includes the separate administration of one of the compounds or agents given first, followed by the second.

In one embodiment, the Therapeutics of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the Therapeutics of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), TR6 (International Publication No. WO 98/30694), OPG, and neutrokine-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

In certain embodiments, Therapeutics of the invention are administered in combination with antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). Non-nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in

combination with the Therapeutics of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (sâquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with Therapeutics of the invention to treat AIDS and/or to prevent or treat HIV infection.

In other embodiments, Therapeutics of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, Therapeutics of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat or prevent an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat or prevent an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, Therapeutics of the invention are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat or prevent an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat or prevent an opportunistic cytomegalovirus infection. In another specific

embodiment, Therapeutics of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat or prevent an opportunistic fungal infection. In another specific embodiment, Therapeutics of the invention are used in any combination with  
5 ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, Therapeutics of the invention are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat or prevent an opportunistic *Toxoplasma gondii* infection. In another specific embodiment,  
10 Therapeutics of the invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat or prevent an opportunistic bacterial infection.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered  
15 with the Therapeutics of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the Therapeutics of the invention include, but are not limited to, amoxicillin,  
20 beta-lactamases, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamthoxazole, and vancomycin.

25 Conventional nonspecific immunosuppressive agents, that may be administered in combination with the Therapeutics of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells.

30 In specific embodiments, Therapeutics of the invention are administered in combination with immunosuppressants. Immunosuppressants preparations that may

be administered with the Therapeutics of the invention include, but are not limited to, ORTHOCLONE™ (OKT3), SANDIMMUNE™/NEORAL™/SANGDYA™ (cyclosporin), PROGRAF™ (tacrolimus), CELLCEPT™ (mycophenolate), Azathioprine, glucorticosteroids, and RAPAMUNE™ (sirolimus). In a specific  
5 embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

In an additional embodiment, Therapeutics of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the  
10 Therapeutics of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, and GAMIMUNE™. In a specific embodiment, Therapeutics of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

15 In an additional embodiment, the Therapeutics of the invention are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the Therapeutics of the invention include, but are not limited to, glucocorticoids and the nonsteroidal anti-inflammatories, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives,  
20 arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal,  
25 pifoxime, proquazone, proxazole, and tenidap.

In another embodiment, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the Therapeutics of the invention include, but are not limited to, antibiotic derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and  
30 dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b, glutamic acid, plicamycin,

mercaptapurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (e.g., medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephallen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids and combinations (e.g., bethamethasone sodium phosphate); and others (e.g., dicarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, and etoposide).

In a specific embodiment, Therapeutics of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or any combination of the components of CHOP. In another embodiment, Therapeutics of the invention are administered in combination with Rituximab. In a further embodiment, Therapeutics of the invention are administered with Rituxmab and CHOP, or Rituxmab and any combination of the components of CHOP.

In an additional embodiment, the Therapeutics of the invention are administered in combination with cytokines. Cytokines that may be administered with the Therapeutics of the invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha. In another embodiment, Therapeutics of the invention may be administered with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21.

In an additional embodiment, the Therapeutics of the invention are administered in combination with angiogenic proteins. Angiogenic proteins that may be administered with the Therapeutics of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as

disclosed in European Patent Number EP-282317; Placental Growth Factor (PIGF), as disclosed in International Publication Number WO 92/06194; Placental Growth Factor-2 (PIGF-2), as disclosed in Hauser et al., Growth Factors, 4:259-268 (1993); Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication  
5 Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2 (VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular Endothelial Growth Factor B (VEGF-3); Vascular Endothelial Growth Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO  
10 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German Patent Number DE19639601. The above mentioned references are incorporated herein by  
15 reference herein.

In an additional embodiment, the Therapeutics of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the Therapeutics of the invention include, but are not limited to, LEUKINE™ (SARGRAMOSTIM™) and  
20 NEUPOGEN™ (FILGRASTIM™).

In an additional embodiment, the Therapeutics of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered with the Therapeutics of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8,  
25 FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

In additional embodiments, the Therapeutics of the invention are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

30 **Example 24: Method of Treating Decreased Levels of the Polypeptide**



The present invention relates to a method for treating an individual in need of an increased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an agonist of the invention (including polypeptides of the invention). Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of a secreted protein in an individual can be treated by administering the polypeptide of the present invention, preferably in the secreted form. Thus, the invention also provides a method of treatment of an individual in need of an increased level of the polypeptide comprising administering to such an individual a Therapeutic comprising an amount of the polypeptide to increase the activity level of the polypeptide in such an individual.

For example, a patient with decreased levels of a polypeptide receives a daily dose 0.1-100 ug/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is in the secreted form. The exact details of the dosing scheme, based on administration and formulation, are provided in Example 23.

#### **Example 25: Method of Treating Increased Levels of the Polypeptide**

The present invention also relates to a method of treating an individual in need of a decreased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an antagonist of the invention (including polypeptides and antibodies of the invention).

In one example, antisense technology is used to inhibit production of a polypeptide of the present invention. This technology is one example of a method of decreasing levels of a polypeptide, preferably a secreted form, due to a variety of etiologies, such as cancer. For example, a patient diagnosed with abnormally increased levels of a polypeptide is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided in Example 23.

**Example 26: Method of Treatment Using Gene Therapy-Ex Vivo**

One method of gene therapy transplants fibroblasts, which are capable of expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37 degree C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

The cDNA encoding a polypeptide of the present invention can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 1 using primers and having appropriate restriction sites and initiation/stop codons, if necessary. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector has the gene of interest properly inserted.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with

10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

5        Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the  
10       media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether protein is  
15       produced.

The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

20       **Example 27: Gene Therapy Using Endogenous Genes Corresponding To Polynucleotides of the Invention**

Another method of gene therapy according to the present invention involves operably associating the endogenous polynucleotide sequence of the invention with a promoter via homologous recombination as described, for example, in U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411,  
25       published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., *Proc. Natl. Acad. Sci. USA*, 86:8932-8935 (1989); and Zijlstra et al., *Nature*, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired.

30       Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous

polynucleotide sequence, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of the polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences can be amplified using PCR. Preferably, the  
5 amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

10 The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size  
15 fractionated on an agarose gel then purified by phenol extraction and ethanol precipitation.

In this Example, the polynucleotide constructs are administered as naked polynucleotides via electroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral  
20 sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous polynucleotide sequence. This results in the expression of polynucleotide corresponding to the  
25 polynucleotide in the cell. Expression may be detected by immunological staining, or any other method known in the art.

Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient  
30 medium. An aliquot of the cell suspension is removed for counting, and the remaining cells are subjected to centrifugation. The supernatant is aspirated and the pellet is

resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM Na<sub>2</sub> HPO<sub>4</sub>, 6 mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains  
5 approximately  $3 \times 10^6$  cells/ml. Electroporation should be performed immediately following resuspension.

Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the locus corresponding to the polynucleotide of the invention, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with  
10 HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3' end. Two non-coding sequences are amplified via PCR: one non-coding sequence (fragment 1) is amplified with a HindIII site at the 5' end and an Xba site at the 3' end; the other non-coding sequence (fragment 2) is amplified with a BamHI site at the 5' end and a HindIII site at the 3' end. The CMV promoter and the  
15 fragments (1 and 2) are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; fragment 1 - XbaI; fragment 2 - BamHI) and ligated together. The resulting ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap  
20 (Bio-Rad). The final DNA concentration is generally at least 120 µg/ml. 0.5 ml of the cell suspension (containing approximately  $1.5 \times 10^6$  cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960 µF and 250-300 V, respectively. As voltage increases, cell survival decreases, but  
25 the percentage of surviving cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.

Electroporated cells are maintained at room temperature for approximately 5 min, and the contents of the cuvette are then gently removed with a sterile transfer  
30 pipette. The cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a 10 cm dish and incubated at 37 degree C. The following

day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

The engineered fibroblasts are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product. The fibroblasts can then be introduced into a patient as described above.

**Example 28: Method of Treatment Using Gene Therapy - In Vivo**

Another aspect of the present invention is using *in vivo* gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) sequences into an animal to increase or decrease the expression of the polypeptide. The polynucleotide of the present invention may be operatively linked to a promoter or any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 5705151, 5580859; Tabata et al., Cardiovasc. Res. 35(3):470-479 (1997); Chao et al., Pharmacol. Res. 35(6):517-522 (1997); Wolff, Neuromuscul. Disord. 7(5):314-318 (1997); Schwartz et al., Gene Ther. 3(5):405-411 (1996); Tsurumi et al., Circulation 94(12):3281-3290 (1996) (incorporated herein by reference).

The polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the present invention may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (1995) Ann. NY Acad. Sci. 772:126-139 and Abdallah B. et al. (1995) Biol. Cell 85(1):1-7) which can be prepared by methods well known to those

skilled in the art.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence

can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or  
5 mucous membranes of the nose. In addition, naked polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected polynucleotide in muscle *in vivo* is determined as follows. Suitable template DNA for production of mRNA coding for polypeptide of the present invention is prepared in accordance with a standard  
10 recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

Five to six week old female and male Balb/C mice are anesthetized by  
15 intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future  
20 localization, and the skin is closed with stainless steel clips.

After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15 um cross-section of the individual quadriceps muscles is histochemically stained for protein expression. A time course for protein expression may be done in a similar fashion except that  
25 quadriceps from different mice are harvested at different times. Persistence of DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be use to extrapolate proper dosages and other treatment parameters in humans and other animals using naked  
30 DNA.

#### **Example 29: Transgenic Animals.**



The polypeptides of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, *e.g.*, baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

Any technique known in the art may be used to introduce the transgene (*i.e.*, polynucleotides of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al., Appl. Microbiol. Biotechnol. 40:691-698 (1994); Carver et al., Biotechnology (NY) 11:1263-1270 (1993); Wright et al., Biotechnology (NY) 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321 (1989)); electroporation of cells or embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, *e.g.*, Ulmer et al., Science 259:1745 (1993); introducing nucleic acid constructs into embryonic pluripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano et al., Cell 57:717-723 (1989); etc. For a review of such techniques, see Gordon, "Transgenic Animals," Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety.

Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)).

The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, *i.e.*, mosaic animals or chimeric. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, *e.g.*, head-to-head tandems or

head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of

heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the  
5 transgene on a distinct background that is appropriate for an experimental model of interest.

Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying conditions and/or disorders associated  
10 with aberrant expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

**Example 30: Knock-Out Animals.**

Endogenous gene expression can also be reduced by inactivating or "knocking  
15 out" the gene and/or its promoter using targeted homologous recombination. (*E.g.*, see Smithies et al., Nature 317:230-234 (1985); Thomas & Capecchi, Cell 51:503-512 (1987); Thompson et al., Cell 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by  
20 DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene  
25 of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (*e.g.*, see Thomas & Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely  
30 adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art.

In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (e.g., knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (i.e., animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

Transgenic and "knock-out" animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying conditions and/or disorders associated with aberrant expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

**Example 31. Isolation of antibody fragments directed against polypeptides of the invention from a library of scFvs.**

Naturally occurring V-genes isolated from human PBLs are constructed into a large library of antibody fragments which contain reactivities against a polypeptide having the amino acid sequence of SEQ ID NO:Y to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein in its entirety by reference).

**Rescue of the library.**

A library of scFvs is constructed from the RNA of human PBLs as described in WO92/01047. To rescue phage displaying antibody fragments, approximately  $10^9$  *E. coli* harboring the phagemid are used to inoculate 50 ml of 2x TY containing 1% glucose and 100 micrograms/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to inoculate 50 ml of 2xTY-AMP-GLU,  $2 \times 10^8$  TU of delta gene 3 helper (M13 delta gene III, see WO92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2x TY containing 100 micrograms/ml ampicillin and 50 micrograms/ml kanamycin and grown overnight. Phage are prepared as described in WO92/01047.

M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis.

The culture is incubated for 1 hour at 37°C without shaking and then for a further hour at 37°C with shaking. Cells were spun down (IEC-Centra 8, 4000 revs/min for 10 min), resuspended in 300 ml 2x TY broth containing 100 micrograms ampicillin/ml and 25 micrograms kanamycin/ml (2x TY-AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 micrometer filter (Minisart NML; Sartorius) to give a final concentration of approximately  $10^{13}$  transducing units/ml (ampicillin-resistant clones).

#### 10 **Panning the Library.**

Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100 micrograms/ml or 10 micrograms/ml of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately  $10^{13}$  TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log *E. coli* TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The *E. coli* are then plated on TYE plates containing 1% glucose and 100 micrograms/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

#### **Characterization of Binders.**

Eluted phage from the third and fourth rounds of selection are used to infect *E. coli* HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtiter plates coated with either

10 picograms/ml of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see e.g., WO92/01047) and then by sequencing.

5 **Example 32: Assays Detecting Stimulation or Inhibition of B cell Proliferation and Differentiation**

Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may  
10 impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL-5, IL-6, IL-7, IL10, IL-13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can,  
15 in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations.

One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune  
20 responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations and their  
25 precursors.

In Vitro Assay- Purified polypeptides of the invention, or truncated forms thereof, is assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of the polypeptides of the invention on purified human tonsillar B cells, measured  
30 qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in

the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220).

Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added  $10^5$  B-cells suspended in culture medium (RPMI 1640 containing 10% FBS,  $5 \times 10^{-5}$ M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and  $10^{-5}$  dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated by a 20h pulse (1uCi/well) with  $^3$ H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

In Vivo Assay- BALB/c mice are injected (i.p.) twice per day with buffer only, or 2 mg/Kg of a polypeptide of the invention, or truncated forms thereof. Mice receive this treatment for 4 consecutive days, at which time they are sacrificed and various tissues and serum collected for analyses. Comparison of H&E sections from normal spleens and spleens treated with polypeptides of the invention identify the results of the activity of the polypeptides on spleen cells, such as the diffusion of peria-  
arterial lymphatic sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-cell regions.

Flow cytometric analyses of the spleens from mice treated with polypeptide is used to indicate whether the polypeptide specifically increases the proportion of ThB+, CD45R(B220)dull B cells over that which is observed in control mice.



Likewise, a predicted consequence of increased mature B-cell representation in vivo is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are compared between buffer and polypeptide-treated mice.

5 The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

### Example 33: T Cell Proliferation Assay

10 A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of  $^3\text{H}$ -thymidine. The assay is performed as follows. Ninety-six well plates are coated with 100  $\mu\text{l}$ /well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched control mAb (B33.1) overnight at 4 degrees C (1  $\mu\text{g}/\text{ml}$  in .05M bicarbonate buffer, pH 9.5), then  
15 washed three times with PBS. PBMC are isolated by F/H gradient centrifugation from human peripheral blood and added to quadruplicate wells ( $5 \times 10^4/\text{well}$ ) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence of varying concentrations of polypeptides of the invention (total volume 200  $\mu\text{l}$ ). Relevant protein buffer and medium alone are controls. After 48 hr. culture at 37 degrees C, plates are spun for 2 min. at 1000  
20 rpm and 100  $\mu\text{l}$  of supernatant is removed and stored -20 degrees C for measurement of IL-2 (or other cytokines) if effect on proliferation is observed. Wells are supplemented with 100  $\mu\text{l}$  of medium containing 0.5 uCi of  $^3\text{H}$ -thymidine and cultured at 37 degrees C for 18-24 hr. Wells are harvested and incorporation of  $^3\text{H}$ -thymidine used as a measure of proliferation. Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is  
25 also used as a control which enhances proliferation. Control antibody which does not induce proliferation of T cells is used as the negative controls for the effects of polypeptides of the invention.

The studies described in this example tested activity of polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to  
30 test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

**Example 34: Effect of Polypeptides of the Invention on the Expression of MHC Class II, Costimulatory and Adhesion Molecules and Cell Differentiation of Monocytes and Monocyte-Derived Human Dendritic Cells**

5

Dendritic cells are generated by the expansion of proliferating precursors found in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for 7-10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and MHC class II antigens). Treatment with activating factors, such as TNF- $\alpha$ , causes a rapid change in surface phenotype (increased expression of MHC class I and II, costimulatory and adhesion molecules, downregulation of FC $\gamma$ RII, upregulation of CD83). These changes correlate with increased antigen-presenting capacity and with functional maturation of the dendritic cells.

15

FACS analysis of surface antigens is performed as follows. Cells are treated 1-3 days with increasing concentrations of polypeptides of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

20

Effect on the production of cytokines. Cytokines generated by dendritic cells, in particular IL-12, are important in the initiation of T-cell dependent immune responses. IL-12 strongly influences the development of Th1 helper T-cell immune response, and induces cytotoxic T and NK cell function. An ELISA is used to measure the IL-12 release as follows. Dendritic cells ( $10^6$ /ml) are treated with increasing concentrations of polypeptides of the invention for 24 hours. LPS (100 ng/ml) is added to the cell culture as positive control. Supernatants from the cell cultures are then collected and analyzed for IL-12 content using commercial ELISA kit (e.g., R & D Systems (Minneapolis, MN)). The standard protocols provided with the kits are used.

25

30

Effect on the expression of MHC Class II, costimulatory and adhesion molecules. Three major families of cell surface antigens can be identified on monocytes: adhesion molecules, molecules involved in antigen presentation, and Fc receptor. Modulation of the expression of MHC class II antigens and other costimulatory molecules, such as B7 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and ability to induce T cell activation. Increase expression of Fc receptors may correlate with improved monocyte cytotoxic activity, cytokine release and phagocytosis.

FACS analysis is used to examine the surface antigens as follows. Monocytes are treated 1-5 days with increasing concentrations of polypeptides of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degreesC. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

Monocyte activation and/or increased survival. Assays for molecules that activate (or alternatively, inactivate) monocytes and/or increase monocyte survival (or alternatively, decrease monocyte survival) are known in the art and may routinely be applied to determine whether a molecule of the invention functions as an inhibitor or activator of monocytes. Polypeptides, agonists, or antagonists of the invention can be screened using the three assays described below. For each of these assays, Peripheral blood mononuclear cells (PBMC) are purified from single donor leukopacks (American Red Cross, Baltimore, MD) by centrifugation through a Histopaque gradient (Sigma). Monocytes are isolated from PBMC by counterflow centrifugal elutriation.

Monocyte Survival Assay. Human peripheral blood monocytes progressively lose viability when cultured in absence of serum or other stimuli. Their death results from internally regulated process (apoptosis). Addition to the culture of activating factors, such as TNF-alpha dramatically improves cell survival and prevents DNA

fragmentation. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in polypropylene tubes in serum-free medium (positive control), in the presence of 100 ng/ml TNF-alpha (negative control), and in the presence of varying concentrations of the compound to be tested.

5 Cells are suspended at a concentration of  $2 \times 10^6$ /ml in PBS containing PI at a final concentration of 5  $\mu$ g/ml, and then incubated at room temperature for 5 minutes before FACScan analysis. PI uptake has been demonstrated to correlate with DNA fragmentation in this experimental paradigm.

10 Effect on cytokine release. An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through the release of cytokines after stimulation. An ELISA to measure cytokine release is performed as follows. Human monocytes are incubated at a density of  $5 \times 10^5$  cells/ml with increasing concentrations of the a polypeptide of the invention and under the  
15 same conditions, but in the absence of the polypeptide. For IL-12 production, the cells are primed overnight with IFN (100 U/ml) in presence of a polypeptide of the invention. LPS (10 ng/ml) is then added. Conditioned media are collected after 24h and kept frozen until use. Measurement of TNF-alpha, IL-10, MCP-1 and IL-8 is then performed using a commercially available ELISA kit (e.g., R & D Systems  
20 (Minneapolis, MN)) and applying the standard protocols provided with the kit.

Oxidative burst. Purified monocytes are plated in 96-w plate at  $2 \times 10^5$  cell/well. Increasing concentrations of polypeptides of the invention are added to the wells in a total volume of 0.2 ml culture medium (RPMI 1640 + 10% FCS, glutamine  
25 and antibiotics). After 3 days incubation, the plates are centrifuged and the medium is removed from the wells. To the macrophage monolayers, 0.2 ml per well of phenol red solution (140 mM NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.56 mM phenol red and 19 U/ml of HRPO) is added, together with the stimulant (200 nM PMA). The plates are incubated at 37°C for 2 hours and the  
30 reaction is stopped by adding 20  $\mu$ l 1N NaOH per well. The absorbance is read at 610

nm. To calculate the amount of  $H_2O_2$  produced by the macrophages, a standard curve of a  $H_2O_2$  solution of known molarity is performed for each experiment.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polypeptides, polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

### **Example 35: Biological Effects of Polypeptides of the Invention**

#### **Astrocyte and Neuronal Assays**

Recombinant polypeptides of the invention, expressed in *Escherichia coli* and purified as described above, can be tested for activity in promoting the survival, neurite outgrowth, or phenotypic differentiation of cortical neuronal cells and for inducing the proliferation of glial fibrillary acidic protein immunopositive cells, astrocytes. The selection of cortical cells for the bioassay is based on the prevalent expression of FGF-1 and FGF-2 in cortical structures and on the previously reported enhancement of cortical neuronal survival resulting from FGF-2 treatment. A thymidine incorporation assay, for example, can be used to elucidate a polypeptide of the invention's activity on these cells.

Moreover, previous reports describing the biological effects of FGF-2 (basic FGF) on cortical or hippocampal neurons *in vitro* have demonstrated increases in both neuron survival and neurite outgrowth (Walicke et al., "Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension." *Proc. Natl. Acad. Sci. USA* 83:3012-3016. (1986), assay herein incorporated by reference in its entirety). However, reports from experiments done on PC-12 cells suggest that these two responses are not necessarily synonymous and may depend on not only which FGF is being tested but also on which receptor(s) are expressed on the target cells. Using the primary cortical neuronal culture paradigm, the ability of a polypeptide of the invention to induce neurite outgrowth can be compared to the response achieved with FGF-2 using, for example, a thymidine incorporation assay.

Fibroblast and endothelial cell assays

Human lung fibroblasts are obtained from Clonetics (San Diego, CA) and maintained in growth media from Clonetics. Dermal microvascular endothelial cells are obtained from Cell Applications (San Diego, CA). For proliferation assays, the human lung fibroblasts and dermal microvascular endothelial cells can be cultured at 5,000 cells/well in a 96-well plate for one day in growth medium. The cells are then incubated for one day in 0.1% BSA basal medium. After replacing the medium with fresh 0.1% BSA medium, the cells are incubated with the test proteins for 3 days. Alamar Blue (Alamar Biosciences, Sacramento, CA) is added to each well to a final concentration of 10%. The cells are incubated for 4 hr. Cell viability is measured by reading in a CytoFluor fluorescence reader. For the PGE<sub>2</sub> assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or polypeptides of the invention with or without IL-1 $\alpha$  for 24 hours. The supernatants are collected and assayed for PGE<sub>2</sub> by EIA kit (Cayman, Ann Arbor, MI). For the IL-6 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or with or without polypeptides of the invention IL-1 $\alpha$  for 24 hours. The supernatants are collected and assayed for IL-6 by ELISA kit (Endogen, Cambridge, MA).

Human lung fibroblasts are cultured with FGF-2 or polypeptides of the invention for 3 days in basal medium before the addition of Alamar Blue to assess effects on growth of the fibroblasts. FGF-2 should show a stimulation at 10 - 2500 ng/ml which can be used to compare stimulation with polypeptides of the invention.

Parkinson Models.

The loss of motor function in Parkinson's disease is attributed to a deficiency of striatal dopamine resulting from the degeneration of the nigrostriatal dopaminergic projection neurons. An animal model for Parkinson's that has been extensively characterized involves the systemic administration of 1-methyl-4 phenyl 1,2,3,6-tetrahydropyridine (MPTP). In the CNS, MPTP is taken-up by astrocytes and catabolized

by monoamine oxidase B to 1-methyl-4-phenyl pyridine (MPP<sup>+</sup>) and released. Subsequently, MPP<sup>+</sup> is actively accumulated in dopaminergic neurons by the high-affinity reuptake transporter for dopamine. MPP<sup>+</sup> is then concentrated in mitochondria by the electrochemical gradient and selectively inhibits nicotinamide adenine disphosphate: ubiquinone oxidoreductionase (complex I), thereby interfering with electron transport and eventually generating oxygen radicals.

It has been demonstrated in tissue culture paradigms that FGF-2 (basic FGF) has trophic activity towards nigral dopaminergic neurons (Ferrari et al., Dev. Biol. 1989). Recently, Dr. Unsicker's group has demonstrated that administering FGF-2 in gel foam implants in the striatum results in the near complete protection of nigral dopaminergic neurons from the toxicity associated with MPTP exposure (Otto and Unsicker, J. Neuroscience, 1990).

Based on the data with FGF-2, polypeptides of the invention can be evaluated to determine whether it has an action similar to that of FGF-2 in enhancing dopaminergic neuronal survival *in vitro* and it can also be tested *in vivo* for protection of dopaminergic neurons in the striatum from the damage associated with MPTP treatment. The potential effect of a polypeptide of the invention is first examined *in vitro* in a dopaminergic neuronal cell culture paradigm. The cultures are prepared by dissecting the midbrain floor plate from gestation day 14 Wistar rat embryos. The tissue is dissociated with trypsin and seeded at a density of 200,000 cells/cm<sup>2</sup> on polyorthinine-laminin coated glass coverslips. The cells are maintained in Dulbecco's Modified Eagle's medium and F12 medium containing hormonal supplements (N1). The cultures are fixed with paraformaldehyde after 8 days *in vitro* and are processed for tyrosine hydroxylase, a specific marker for dopaminergic neurons, immunohistochemical staining. Dissociated cell cultures are prepared from embryonic rats. The culture medium is changed every third day and the factors are also added at that time.

Since the dopaminergic neurons are isolated from animals at gestation day 14, a developmental time which is past the stage when the dopaminergic precursor cells are proliferating, an increase in the number of tyrosine hydroxylase immunopositive neurons would represent an increase in the number of dopaminergic neurons surviving *in vitro*.

Therefore, if a polypeptide of the invention acts to prolong the survival of dopaminergic neurons, it would suggest that the polypeptide may be involved in Parkinson's Disease.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to  
5 test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 36: The Effect of Polypeptides of the Invention on the Growth of Vascular Endothelial Cells**

10

On day 1, human umbilical vein endothelial cells (HUVEC) are seeded at  $2-5 \times 10^4$  cells/35 mm dish density in M199 medium containing 4% fetal bovine serum (FBS), 16 units/ml heparin, and 50 units/ml endothelial cell growth supplements (ECGS, Biotechnology, Inc.). On day 2, the medium is replaced with M199 containing 10% FBS, 8  
15 units/ml heparin. A polypeptide having the amino acid sequence of SEQ ID NO:Y, and positive controls, such as VEGF and basic FGF (bFGF) are added, at varying concentrations. On days 4 and 6, the medium is replaced. On day 8, cell number is determined with a Coulter Counter.

An increase in the number of HUVEC cells indicates that the polypeptide of the  
20 invention may proliferate vascular endothelial cells.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the  
25 invention.

**Example 37: Stimulatory Effect of Polypeptides of the Invention on the Proliferation of Vascular Endothelial Cells**

For evaluation of mitogenic activity of growth factors, the colorimetric MTS  
30 (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)2H-tetrazolium) assay with the electron coupling reagent PMS (phenazine methosulfate) was performed (CellTiter 96 AQ, Promega). Cells are seeded in a 96-well plate (5,000



cells/well) in 0.1 mL serum-supplemented medium and are allowed to attach overnight. After serum-starvation for 12 hours in 0.5% FBS, conditions (bFGF, VEGF<sub>165</sub> or a polypeptide of the invention in 0.5% FBS) with or without Heparin (8 U/ml) are added to wells for 48 hours. 20 mg of MTS/PMS mixture (1:0.05) are added per well and allowed to incubate for 1 hour at 37°C before measuring the absorbance at 490 nm in an ELISA plate reader. Background absorbance from control wells (some media, no cells) is subtracted, and seven wells are performed in parallel for each condition. See, Leak *et al.* *In Vitro Cell. Dev. Biol.* 30A:512-518 (1994).

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 38: Inhibition of PDGF-induced Vascular Smooth Muscle Cell Proliferation Stimulatory Effect**

HAoSMC proliferation can be measured, for example, by BrdUrd incorporation. Briefly, subconfluent, quiescent cells grown on the 4-chamber slides are transfected with CRP or FITC-labeled AT2-3LP. Then, the cells are pulsed with 10% calf serum and 6 mg/ml BrdUrd. After 24 h, immunocytochemistry is performed by using BrdUrd Staining Kit (Zymed Laboratories). In brief, the cells are incubated with the biotinylated mouse anti-BrdUrd antibody at 4 degrees C for 2 h after being exposed to denaturing solution and then incubated with the streptavidin-peroxidase and diaminobenzidine. After counterstaining with hematoxylin, the cells are mounted for microscopic examination, and the BrdUrd-positive cells are counted. The BrdUrd index is calculated as a percent of the BrdUrd-positive cells to the total cell number. In addition, the simultaneous detection of the BrdUrd staining (nucleus) and the FITC uptake (cytoplasm) is performed for individual cells by the concomitant use of bright field illumination and dark field-UV fluorescent illumination. See, Hayashida *et al.*, *J. Biol. Chem.* 6:271(36):21985-21992 (1996).

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

5

### **Example 39: Stimulation of Endothelial Migration**

This example will be used to explore the possibility that a polypeptide of the invention may stimulate lymphatic endothelial cell migration.

10 Endothelial cell migration assays are performed using a 48 well microchemotaxis chamber (Neuroprobe Inc., Cabin John, MD; Falk, W., et al., J. Immunological Methods 1980;33:239-247). Polyvinylpyrrolidone-free polycarbonate filters with a pore size of 8  
15  $\mu\text{m}$  (Nucleopore Corp. Cambridge, MA) are coated with 0.1% gelatin for at least 6 hours at room temperature and dried under sterile air. Test substances are diluted to appropriate concentrations in M199 supplemented with 0.25% bovine serum albumin (BSA), and 25  
20  $\mu\text{l}$  of the final dilution is placed in the lower chamber of the modified Boyden apparatus. Subconfluent, early passage (2-6) HUVEC or BMEC cultures are washed and trypsinized for the minimum time required to achieve cell detachment. After placing the filter between lower and upper chamber,  $2.5 \times 10^5$  cells suspended in 50  $\mu\text{l}$  M199 containing 1%  
25 FBS are seeded in the upper compartment. The apparatus is then incubated for 5 hours at  $37^\circ\text{C}$  in a humidified chamber with 5%  $\text{CO}_2$  to allow cell migration. After the incubation period, the filter is removed and the upper side of the filter with the non-migrated cells is scraped with a rubber policeman. The filters are fixed with methanol and stained with a Giemsa solution (Diff-Quick, Baxter, McGraw Park, IL). Migration is quantified by  
counting cells of three random high-power fields (40x) in each well, and all groups are performed in quadruplicate.

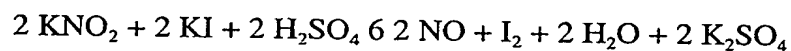
The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the  
30 invention.

**Example 40: Stimulation of Nitric Oxide Production by Endothelial Cells**

Nitric oxide released by the vascular endothelium is believed to be a mediator of vascular endothelium relaxation. Thus, activity of a polypeptide of the invention can be assayed by determining nitric oxide production by endothelial cells in response to the polypeptide.

Nitric oxide is measured in 96-well plates of confluent microvascular endothelial cells after 24 hours starvation and a subsequent 4 hr exposure to various levels of a positive control (such as VEGF-1) and the polypeptide of the invention. Nitric oxide in the medium is determined by use of the Griess reagent to measure total nitrite after reduction of nitric oxide-derived nitrate by nitrate reductase. The effect of the polypeptide of the invention on nitric oxide release is examined on HUVEC.

Briefly, NO release from cultured HUVEC monolayer is measured with a NO-specific polarographic electrode connected to a NO meter (Iso-NO, World Precision Instruments Inc.) (1049). Calibration of the NO elements is performed according to the following equation:



The standard calibration curve is obtained by adding graded concentrations of  $\text{KNO}_2$  (0, 5, 10, 25, 50, 100, 250, and 500 nmol/L) into the calibration solution containing KI and  $\text{H}_2\text{SO}_4$ . The specificity of the Iso-NO electrode to NO is previously determined by measurement of NO from authentic NO gas (1050). The culture medium is removed and HUVECs are washed twice with Dulbecco's phosphate buffered saline. The cells are then bathed in 5 ml of filtered Krebs-Henseleit solution in 6-well plates, and the cell plates are kept on a slide warmer (Lab Line Instruments Inc.) To maintain the temperature at 37°C. The NO sensor probe is inserted vertically into the wells, keeping the tip of the electrode 2 mm under the surface of the solution, before addition of the different conditions. S-nitroso acetyl penicillamin (SNAP) is used as a positive control. The amount of released NO is expressed as picomoles per  $1 \times 10^6$  endothelial cells. All values reported are means of four to six measurements in each group (number of cell culture wells). See, Leak *et al. Biochem. and Biophys. Res. Comm.* 217:96-105 (1995).

The studies described in this example tested activity of polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

5

**Example 41: Effect of Polypeptides of the Invention on Cord Formation in Angiogenesis**

Another step in angiogenesis is cord formation, marked by differentiation of endothelial cells. This bioassay measures the ability of microvascular endothelial cells to form capillary-like structures (hollow structures) when cultured *in vitro*.

CADMEC (microvascular endothelial cells) are purchased from Cell Applications, Inc. as proliferating (passage 2) cells and are cultured in Cell Applications' CADMEC Growth Medium and used at passage 5. For the *in vitro* angiogenesis assay, the wells of a 48-well cell culture plate are coated with Cell Applications' Attachment Factor Medium (200 ml/well) for 30 min. at 37°C. CADMEC are seeded onto the coated wells at 7,500 cells/well and cultured overnight in Growth Medium. The Growth Medium is then replaced with 300 mg Cell Applications' Chord Formation Medium containing control buffer or a polypeptide of the invention (0.1 to 100 ng/ml) and the cells are cultured for an additional 48 hr. The numbers and lengths of the capillary-like chords are quantitated through use of the Boeckeler VIA-170 video image analyzer. All assays are done in triplicate.

Commercial (R&D) VEGF (50 ng/ml) is used as a positive control. b-esteradiol (1 ng/ml) is used as a negative control. The appropriate buffer (without protein) is also utilized as a control.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

30

**Example 42: Angiogenic Effect on Chick Chorioallantoic Membrane**

Chick chorioallantoic membrane (CAM) is a well-established system to examine angiogenesis. Blood vessel formation on CAM is easily visible and quantifiable. The ability of polypeptides of the invention to stimulate angiogenesis in CAM can be examined.

Fertilized eggs of the White Leghorn chick (*Gallus gallus*) and the Japanese quail (*Coturnix coturnix*) are incubated at 37.8°C and 80% humidity. Differentiated CAM of 16-day-old chick and 13-day-old quail embryos is studied with the following methods.

On Day 4 of development, a window is made into the egg shell of chick eggs. The embryos are checked for normal development and the eggs sealed with cellotape. They are further incubated until Day 13. Thermanox coverslips (Nunc, Naperville, IL) are cut into disks of about 5 mm in diameter. Sterile and salt-free growth factors are dissolved in distilled water and about 3.3 mg/ 5 ml are pipetted on the disks. After air-drying, the inverted disks are applied on CAM. After 3 days, the specimens are fixed in 3% glutaraldehyde and 2% formaldehyde and rinsed in 0.12 M sodium cacodylate buffer. They are photographed with a stereo microscope [Wild M8] and embedded for semi- and ultrathin sectioning as described above. Controls are performed with carrier disks alone.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 43: Angiogenesis Assay Using a Matrigel Implant in Mouse**

*In vivo* angiogenesis assay of a polypeptide of the invention measures the ability of an existing capillary network to form new vessels in an implanted capsule of murine extracellular matrix material (Matrigel). The protein is mixed with the liquid Matrigel at 4 degree C and the mixture is then injected subcutaneously in mice where it solidifies. After 7 days, the solid "plug" of Matrigel is removed and examined for the presence of new

blood vessels. Matrigel is purchased from Becton Dickinson Labware/Collaborative Biomedical Products.

When thawed at 4 degree C the Matrigel material is a liquid. The Matrigel is mixed with a polypeptide of the invention at 150 ng/ml at 4 degrees C and drawn into cold  
5 3 ml syringes. Female C57Bl/6 mice approximately 8 weeks old are injected with the mixture of Matrigel and experimental protein at 2 sites at the midventral aspect of the abdomen (0.5 ml/site). After 7 days, the mice are sacrificed by cervical dislocation, the Matrigel plugs are removed and cleaned (i.e., all clinging membranes and fibrous tissue is removed). Replicate whole plugs are fixed in neutral buffered 10% formaldehyde,  
10 embedded in paraffin and used to produce sections for histological examination after staining with Masson's Trichrome. Cross sections from 3 different regions of each plug are processed. Selected sections are stained for the presence of vWF. The positive control for this assay is bovine basic FGF (150 ng/ml). Matrigel alone is used to determine basal levels of angiogenesis.

15 The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### 20 **Example 44: Rescue of Ischemia in Rabbit Lower Limb Model**

To study the in vivo effects of polynucleotides and polypeptides of the invention on ischemia, a rabbit hindlimb ischemia model is created by surgical removal of one femoral arteries as described previously (Takeshita *et al.*, *Am J. Pathol* 147:1649-1660  
25 (1995)). The excision of the femoral artery results in retrograde propagation of thrombus and occlusion of the external iliac artery. Consequently, blood flow to the ischemic limb is dependent upon collateral vessels originating from the internal iliac artery (Takeshita *et al.* *Am J. Pathol* 147:1649-1660 (1995)). An interval of 10 days is allowed for post-operative recovery of rabbits and development of endogenous collateral vessels. At 10  
30 day post-operatively (day 0), after performing a baseline angiogram, the internal iliac artery of the ischemic limb is transfected with 500 mg naked expression plasmid

containing a polynucleotide of the invention by arterial gene transfer technology using a hydrogel-coated balloon catheter as described (Riessen *et al. Hum Gene Ther.* 4:749-758 (1993); Leclerc *et al. J. Clin. Invest.* 90: 936-944 (1992)). When a polypeptide of the invention is used in the treatment, a single bolus of 500 mg polypeptide of the invention or control is delivered into the internal iliac artery of the ischemic limb over a period of 1 min. through an infusion catheter. On day 30, various parameters are measured in these rabbits: (a) BP ratio - The blood pressure ratio of systolic pressure of the ischemic limb to that of normal limb; (b) Blood Flow and Flow Reserve - Resting FL: the blood flow during undilated condition and Max FL: the blood flow during fully dilated condition (also an indirect measure of the blood vessel amount) and Flow Reserve is reflected by the ratio of max FL: resting FL; (c) Angiographic Score - This is measured by the angiogram of collateral vessels. A score is determined by the percentage of circles in an overlaying grid that with crossing opacified arteries divided by the total number in the rabbit thigh; (d) Capillary density - The number of collateral capillaries determined in light microscopic sections taken from hindlimbs.

The studies described in this example tested activity of polynucleotides and polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the agonists, and/or antagonists of the invention.

#### **Example 45: Effect of Polypeptides of the Invention on Vasodilation**

Since dilation of vascular endothelium is important in reducing blood pressure, the ability of polypeptides of the invention to affect the blood pressure in spontaneously hypertensive rats (SHR) is examined. Increasing doses (0, 10, 30, 100, 300, and 900 mg/kg) of the polypeptides of the invention are administered to 13-14 week old spontaneously hypertensive rats (SHR). Data are expressed as the mean  $\pm$  SEM. Statistical analysis are performed with a paired t-test and statistical significance is defined as  $p < 0.05$  vs. the response to buffer alone.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to

test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 46: Rat Ischemic Skin Flap Model**

5

The evaluation parameters include skin blood flow, skin temperature, and factor VIII immunohistochemistry or endothelial alkaline phosphatase reaction. Expression of polypeptides of the invention, during the skin ischemia, is studied using in situ hybridization.

10

The study in this model is divided into three parts as follows:

- a) Ischemic skin
- b) Ischemic skin wounds
- c) Normal wounds

The experimental protocol includes:

15

a) Raising a 3x4 cm, single pedicle full-thickness random skin flap (myocutaneous flap over the lower back of the animal).

b) An excisional wounding (4-6 mm in diameter) in the ischemic skin (skin-flap).

c) Topical treatment with a polypeptide of the invention of the excisional wounds (day 0, 1, 2, 3, 4 post-wounding) at the following various dosage ranges: 1mg to 100 mg.

20

d) Harvesting the wound tissues at day 3, 5, 7, 10, 14 and 21 post-wounding for histological, immunohistochemical, and in situ studies.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

25

**Example 47: Peripheral Arterial Disease Model**



Angiogenic therapy using a polypeptide of the invention is a novel therapeutic strategy to obtain restoration of blood flow around the ischemia in case of peripheral arterial diseases. The experimental protocol includes:

- a) One side of the femoral artery is ligated to create ischemic muscle of the hindlimb, the other side of hindlimb serves as a control.
- b) a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-3 weeks.
- c) The ischemic muscle tissue is collected after ligation of the femoral artery at 1, 2, and 3 weeks for the analysis of expression of a polypeptide of the invention and histology. Biopsy is also performed on the other side of normal muscle of the contralateral hindlimb.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 48: Ischemic Myocardial Disease Model**

A polypeptide of the invention is evaluated as a potent mitogen capable of stimulating the development of collateral vessels, and restructuring new vessels after coronary artery occlusion. Alteration of expression of the polypeptide is investigated in situ. The experimental protocol includes:

- a) The heart is exposed through a left-side thoracotomy in the rat. Immediately, the left coronary artery is occluded with a thin suture (6-0) and the thorax is closed.
- b) a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-4 weeks.
- c) Thirty days after the surgery, the heart is removed and cross-sectioned for morphometric and in situ analyzes.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

5

**Example 49: Rat Corneal Wound Healing Model**

This animal model shows the effect of a polypeptide of the invention on neovascularization. The experimental protocol includes:

- 10           a)     Making a 1-1.5 mm long incision from the center of cornea into the stromal layer.
- b)     Inserting a spatula below the lip of the incision facing the outer corner of the eye.
- c)     Making a pocket (its base is 1-1.5 mm from the edge of the eye).
- 15           d)     Positioning a pellet, containing 50ng- 5ug of a polypeptide of the invention, within the pocket.
- e)     Treatment with a polypeptide of the invention can also be applied topically to the corneal wounds in a dosage range of 20mg - 500mg (daily treatment for five days).

20     The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

25     **Example 50: Diabetic Mouse and Glucocorticoid-Impaired Wound Healing Models**

          A.     *Diabetic db+/db+ Mouse Model.*

          To demonstrate that a polypeptide of the invention accelerates the healing process, the genetically diabetic mouse model of wound healing is used. The full thickness wound healing model in the db+/db+ mouse is a well characterized, clinically relevant and reproducible model of impaired wound healing. Healing of the diabetic wound is

30

dependent on formation of granulation tissue and re-epithelialization rather than contraction (Gartner, M.H. *et al.*, *J. Surg. Res.* 52:389 (1992); Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)).

The diabetic animals have many of the characteristic features observed in Type II  
5 diabetes mellitus. Homozygous (db+/db+) mice are obese in comparison to their normal  
heterozygous (db+/+m) littermates. Mutant diabetic (db+/db+) mice have a single  
autosomal recessive mutation on chromosome 4 (db+) (Coleman *et al.* *Proc. Natl. Acad.*  
*Sci. USA* 77:283-293 (1982)). Animals show polyphagia, polydipsia and polyuria.  
Mutant diabetic mice (db+/db+) have elevated blood glucose, increased or normal insulin  
10 levels, and suppressed cell-mediated immunity (Mandel *et al.*, *J. Immunol.* 120:1375  
(1978); Debray-Sachs, M. *et al.*, *Clin. Exp. Immunol.* 51(1):1-7 (1983); Leiter *et al.*, *Am.*  
*J. of Pathol.* 114:46-55 (1985)). Peripheral neuropathy, myocardial complications, and  
microvascular lesions, basement membrane thickening and glomerular filtration  
abnormalities have been described in these animals (Norido, F. *et al.*, *Exp. Neurol.*  
15 83(2):221-232 (1984); Robertson *et al.*, *Diabetes* 29(1):60-67 (1980); Giacomelli *et al.*,  
*Lab Invest.* 40(4):460-473 (1979); Coleman, D.L., *Diabetes* 31 (Suppl):1-6 (1982)). These  
homozygous diabetic mice develop hyperglycemia that is resistant to insulin analogous to  
human type II diabetes (Mandel *et al.*, *J. Immunol.* 120:1375-1377 (1978)).

The characteristics observed in these animals suggests that healing in this model  
20 may be similar to the healing observed in human diabetes (Greenhalgh, *et al.*, *Am. J. of*  
*Pathol.* 136:1235-1246 (1990)).

Genetically diabetic female C57BL/KsJ (db+/db+) mice and their non-diabetic  
(db+/+m) heterozygous littermates are used in this study (Jackson Laboratories). The  
animals are purchased at 6 weeks of age and are 8 weeks old at the beginning of the study.  
25 Animals are individually housed and received food and water ad libitum. All  
manipulations are performed using aseptic techniques. The experiments are conducted  
according to the rules and guidelines of Human Genome Sciences, Inc. Institutional  
Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory  
Animals.

30 Wounding protocol is performed according to previously reported methods  
(Tsuboi, R. and Rifkin, D.B., *J. Exp. Med.* 172:245-251 (1990)). Briefly, on the day of

wounding, animals are anesthetized with an intraperitoneal injection of Avertin (0.01 mg/mL), 2,2,2-tribromoethanol and 2-methyl-2-butanol dissolved in deionized water. The dorsal region of the animal is shaved and the skin washed with 70% ethanol solution and iodine. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is then created using a Keyes tissue punch. Immediately following wounding, the surrounding skin is gently stretched to eliminate wound expansion. The wounds are left open for the duration of the experiment. Application of the treatment is given topically for 5 consecutive days commencing on the day of wounding. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of surgery and at two day intervals thereafter. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

A polypeptide of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology and immunohistochemistry. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

Three groups of 10 animals each (5 diabetic and 5 non-diabetic controls) are evaluated: 1) Vehicle placebo control, 2) untreated group, and 3) treated group.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total square area of the wound. Contraction is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm<sup>2</sup>, the corresponding size of the dermal punch. Calculations are made using the following formula:

$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using a Reichert-Jung microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds are used to assess whether the healing process and the morphologic appearance of the repaired skin is altered by treatment with a polypeptide of the invention. This assessment included verification of the presence of cell accumulation, inflammatory cells, capillaries, fibroblasts, re-epithelialization and epidermal maturity (Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)). A calibrated lens micrometer is used by a blinded observer.

Tissue sections are also stained immunohistochemically with a polyclonal rabbit anti-human keratin antibody using ABC Elite detection system. Human skin is used as a positive tissue control while non-immune IgG is used as a negative control. Keratinocyte growth is determined by evaluating the extent of reepithelialization of the wound using a calibrated lens micrometer.

Proliferating cell nuclear antigen/cyclin (PCNA) in skin specimens is demonstrated by using anti-PCNA antibody (1:50) with an ABC Elite detection system. Human colon cancer can serve as a positive tissue control and human brain tissue can be used as a negative tissue control. Each specimen includes a section with omission of the primary antibody and substitution with non-immune mouse IgG. Ranking of these sections is based on the extent of proliferation on a scale of 0-8, the lower side of the scale reflecting slight proliferation to the higher side reflecting intense proliferation.

Experimental data are analyzed using an unpaired t test. A p value of  $< 0.05$  is considered significant.

### **B. Steroid Impaired Rat Model**

The inhibition of wound healing by steroids has been well documented in various *in vitro* and *in vivo* systems (Wahl, Glucocorticoids and Wound healing. In: Anti-Inflammatory Steroid Action: Basic and Clinical Aspects. 280-302 (1989); Wahlet *et al.*, *J. Immunol.* 115: 476-481 (1975); Werb *et al.*, *J. Exp. Med.* 147:1684-1694 (1978)).

Glucocorticoids retard wound healing by inhibiting angiogenesis, decreasing vascular permeability (Ebert *et al.*, *An. Intern. Med.* 37:701-705 (1952)), fibroblast proliferation,

and collagen synthesis (Beck *et al.*, *Growth Factors*. 5: 295-304 (1991); Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978)) and producing a transient reduction of circulating monocytes (Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl, "Glucocorticoids and wound healing", *In: Antiinflammatory Steroid Action: Basic and Clinical Aspects*, Academic Press, New York, pp. 280-302 (1989)). The systemic administration of steroids to impaired wound healing is a well establish phenomenon in rats (Beck *et al.*, *Growth Factors*. 5: 295-304 (1991); Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl, "Glucocorticoids and wound healing", *In: Antiinflammatory Steroid Action: Basic and Clinical Aspects*, Academic Press, New York, pp. 280-302 (1989); Pierce *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 2229-2233 (1989)).

To demonstrate that a polypeptide of the invention can accelerate the healing process, the effects of multiple topical applications of the polypeptide on full thickness excisional skin wounds in rats in which healing has been impaired by the systemic administration of methylprednisolone is assessed.

Young adult male Sprague Dawley rats weighing 250-300 g (Charles River Laboratories) are used in this example. The animals are purchased at 8 weeks of age and are 9 weeks old at the beginning of the study. The healing response of rats is impaired by the systemic administration of methylprednisolone (17mg/kg/rat intramuscularly) at the time of wounding. Animals are individually housed and received food and water *ad libitum*. All manipulations are performed using aseptic techniques. This study is conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

The wounding protocol is followed according to section A, above. On the day of wounding, animals are anesthetized with an intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The dorsal region of the animal is shaved and the skin washed with 70% ethanol and iodine solutions. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is created using a Keyes tissue punch. The wounds are left open for the duration of the experiment. Applications of the testing materials are given topically once a day for 7 consecutive days commencing on the

day of wounding and subsequent to methylprednisolone administration. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of wounding and at the end of treatment. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

The polypeptide of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

Four groups of 10 animals each (5 with methylprednisolone and 5 without glucocorticoid) are evaluated: 1) Untreated group 2) Vehicle placebo control 3) treated groups.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total area of the wound. Closure is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm<sup>2</sup>, the corresponding size of the dermal punch. Calculations are made using the following formula:

$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using an Olympus microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds allows assessment of whether the healing process and the morphologic appearance of the repaired skin is improved by

treatment with a polypeptide of the invention. A calibrated lens micrometer is used by a blinded observer to determine the distance of the wound gap.

Experimental data are analyzed using an unpaired t test. A p value of  $< 0.05$  is considered significant.

5       The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### 10    Example 51: Lymphadema Animal Model

or       The purpose of this experimental approach is to create an appropriate and consistent lymphedema model for testing the therapeutic effects of a polypeptide of the invention in lymphangiogenesis and re-establishment of the lymphatic circulatory system  
15    in the rat hind limb. Effectiveness is measured by swelling volume of the affected limb, quantification of the amount of lymphatic vasculature, total blood plasma protein, and histopathology. Acute lymphedema is observed for 7-10 days. Perhaps more importantly, the chronic progress of the edema is followed for up to 3-4 weeks.

      Prior to beginning surgery, blood sample is drawn for protein concentration  
20    analysis. Male rats weighing approximately ~350g are dosed with Pentobarbital. Subsequently, the right legs are shaved from knee to hip. The shaved area is swabbed with gauze soaked in 70% EtOH. Blood is drawn for serum total protein testing. Circumference and volumetric measurements are made prior to injecting dye into paws after marking 2 measurement levels (0.5 cm above heel, at mid-pt of dorsal paw). The  
25    intradermal dorsum of both right and left paws are injected with 0.05 ml of 1% Evan's Blue. Circumference and volumetric measurements are then made following injection of dye into paws.

      Using the knee joint as a landmark, a mid-leg inguinal incision is made circumferentially allowing the femoral vessels to be located. Forceps and hemostats are  
30    used to dissect and separate the skin flaps. After locating the femoral vessels, the



lymphatic vessel that runs along side and underneath the vessel(s) is located. The main lymphatic vessels in this area are then electrically coagulated suture ligated.

Using a microscope, muscles in back of the leg (near the semitendinosus and adductors) are bluntly dissected. The popliteal lymph node is then located. The 2 proximal and 2 distal lymphatic vessels and distal blood supply of the popliteal node are then and ligated by suturing. The popliteal lymph node, and any accompanying adipose tissue, is then removed by cutting connective tissues.

Care is taken to control any mild bleeding resulting from this procedure. After lymphatics are occluded, the skin flaps are sealed by using liquid skin (Vetbond) (AJ Buck). The separated skin edges are sealed to the underlying muscle tissue while leaving a gap of ~0.5 cm around the leg. Skin also may be anchored by suturing to underlying muscle when necessary.

To avoid infection, animals are housed individually with mesh (no bedding). Recovering animals are checked daily through the optimal edematous peak, which typically occurred by day 5-7. The plateau edematous peak are then observed. To evaluate the intensity of the lymphedema, the circumference and volumes of 2 designated places on each paw before operation and daily for 7 days are measured. The effect plasma proteins on lymphedema is determined and whether protein analysis is a useful testing perimeter is also investigated. The weights of both control and edematous limbs are evaluated at 2 places. Analysis is performed in a blind manner.

**Circumference Measurements:** Under brief gas anesthetic to prevent limb movement, a cloth tape is used to measure limb circumference. Measurements are done at the ankle bone and dorsal paw by 2 different people then those 2 readings are averaged. Readings are taken from both control and edematous limbs.

**Volumetric Measurements:** On the day of surgery, animals are anesthetized with Pentobarbital and are tested prior to surgery. For daily volumetrics animals are under brief halothane anesthetic (rapid immobilization and quick recovery), both legs are shaved and equally marked using waterproof marker on legs. Legs are first dipped in water, then dipped into instrument to each marked level then measured by Buxco edema software(Chen/Victor). Data is recorded by one person, while the other is dipping the limb to marked area.

Blood-plasma protein measurements: Blood is drawn, spun, and serum separated prior to surgery and then at conclusion for total protein and Ca<sup>2+</sup> comparison.

Limb Weight Comparison: After drawing blood, the animal is prepared for tissue collection. The limbs are amputated using a quillitine, then both experimental and control  
5 legs are cut at the ligature and weighed. A second weighing is done as the tibio-cacaneal joint is disarticulated and the foot is weighed.

Histological Preparations: The transverse muscle located behind the knee (popliteal) area is dissected and arranged in a metal mold, filled with freezeGel, dipped into cold methylbutane, placed into labeled sample bags at - 80EC until sectioning. Upon  
10 sectioning, the muscle is observed under fluorescent microscopy for lymphatics..

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the  
15 invention.

**Example 52: Suppression of TNF alpha-induced adhesion molecule expression by a Polypeptide of the Invention**

The recruitment of lymphocytes to areas of inflammation and angiogenesis  
20 involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and  
25 endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Tumor necrosis factor alpha (TNF- $\alpha$ ), a potent proinflammatory cytokine, is a stimulator of all three CAMs on endothelial cells and may be involved in a wide variety of inflammatory responses, often resulting in a pathological outcome.

The potential of a polypeptide of the invention to mediate a suppression of TNF- $\alpha$  induced CAM expression can be examined. A modified ELISA assay which uses ECs as a solid phase absorbent is employed to measure the amount of CAM expression on TNF- $\alpha$  treated ECs when co-stimulated with a member of the FGF family of proteins.

To perform the experiment, human umbilical vein endothelial cell (HUVEC) cultures are obtained from pooled cord harvests and maintained in growth medium (EGM-2; Clonetics, San Diego, CA) supplemented with 10% FCS and 1% penicillin/streptomycin in a 37 degree C humidified incubator containing 5% CO<sub>2</sub>. HUVECs are seeded in 96-well plates at concentrations of  $1 \times 10^4$  cells/well in EGM medium at 37 degree C for 18-24 hrs or until confluent. The monolayers are subsequently washed 3 times with a serum-free solution of RPMI-1640 supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin, and treated with a given cytokine and/or growth factor(s) for 24 h at 37 degree C. Following incubation, the cells are then evaluated for CAM expression.

Human Umbilical Vein Endothelial cells (HUVECs) are grown in a standard 96 well plate to confluence. Growth medium is removed from the cells and replaced with 90  $\mu$ l of 199 Medium (10% FBS). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10  $\mu$ l volumes). Plates are incubated at 37 degree C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100  $\mu$ l of 0.1% paraformaldehyde-PBS(with Ca<sup>++</sup> and Mg<sup>++</sup>) is added to each well. Plates are held at 4°C for 30 min.

Fixative is then removed from the wells and wells are washed 1X with PBS(+Ca,Mg)+0.5% BSA and drained. Do not allow the wells to dry. Add 10  $\mu$ l of diluted primary antibody to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10  $\mu$ g/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA.

Then add 20  $\mu$ l of diluted ExtrAvidin-Alkaline Phosphotase (1:5,000 dilution) to each well and incubated at 37°C for 30 min. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA. 1 tablet of p-Nitrophenol Phosphate pNPP is dissolved in 5 ml of glycine buffer (pH 10.4). 100  $\mu$ l of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphotase in glycine buffer: 1:5,000 ( $10^0$ ) >  $10^{-0.5}$  >  $10^{-1}$  >  $10^{-1.5}$ . 5  $\mu$ l of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100  $\mu$ l of pNPP reagent must then be added to each of the standard wells. The plate must be incubated at 37°C for 4h. A volume of 50  $\mu$ l of 3M NaOH is added to all wells. The results are quantified on a plate reader at 405 nm. The background subtraction option is used on blank wells filled with glycine buffer only. The template is set up to indicate the concentration of AP-conjugate in each standard well [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

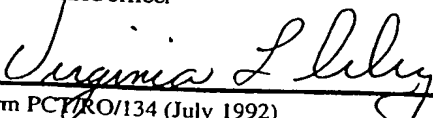
The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference. Further, the hard copy of the sequence listing submitted herewith and the corresponding computer readable form are both incorporated herein by reference in their entireties.

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

|  |   |
|--|---|
| <b>A.</b> The indications made below relate to the microorganism referred to in the description<br>on page <u>90</u> , line <u>n/a</u>   |   |
| <b>B. IDENTIFICATION OF DEPOSIT</b> <div style="text-align: right;">Further deposits are identified on an additional sheet <input type="checkbox"/></div>  |   |
| Name of depositary institution <u>American Type Culture Collection</u>   |   |
| Address of depositary institution (including postal code and country)<br><u>10801 University Boulevard</u><br><u>Manassas, Virginia 20110-2209</u><br><u>United States of America</u>  |   |
| Date of deposit<br><u>September 9, 1998</u>  | Accession Number<br><u>203181</u>   |
| <b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) <div style="text-align: right;">This information is continued on an additional sheet <input type="checkbox"/></div>   |   |
|  |   |
| <b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)  |   |
| <u>Europe</u><br>In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). |   |
| <b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)   |   |
| The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")   |   |
|  |   |
| <div style="text-align: right;">For receiving Office use only</div> <div style="border: 1px solid black; padding: 5px;"><input checked="" type="checkbox"/> This sheet was received with the international application</div> <div style="border: 1px solid black; padding: 5px; margin-top: 10px;">Authorized officer<br/></div>                                | <div style="text-align: right;">For International Bureau use only</div> <div style="border: 1px solid black; padding: 5px;"><input type="checkbox"/> This sheet was received by the International Bureau on:</div> <div style="border: 1px solid black; padding: 5px; margin-top: 10px;">Authorized officer</div> |

ATCC Deposit No. 203181

#### CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

#### NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

#### AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

#### FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

#### UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No. 203181**

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

|   |   |
|---|---|
| <b>A.</b> The indications made below relate to the microorganism referred to in the description<br>on page <u>92</u> , line <u>n/a</u>  |   |
| <b>B. IDENTIFICATION OF DEPOSIT</b><br>Further deposits are identified on an additional sheet <input type="checkbox"/>  |   |
| Name of depositary institution <u>American Type Culture Collection</u>  |   |
| Address of depositary institution (including postal code and country)<br><u>10801 University Boulevard</u><br><u>Manassas, Virginia 20110-2209</u><br><u>United States of America</u>   |   |
| Date of deposit<br><u>October 8, 1998</u>   | Accession Number<br><u>203331</u>   |
| <b>C. ADDITIONAL INDICATIONS</b> (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>  |   |
| <br><br><br><br><br>  |   |
| <b>D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (if the indications are not for all designated States)<br><u>Europe</u><br>In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC). |   |
| <b>E. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)<br>The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")<br><br><br><br><br>  |   |
| <input checked="" type="checkbox"/> For receiving Office use only<br>This sheet was received with the international application<br><br>Authorized officer<br><u>Virginia L. Hiley</u>   | <input type="checkbox"/> For International Bureau use only<br>This sheet was received by the International Bureau on:<br><br>Authorized officer |



**ATCC Deposit No. 203331**

## **CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

## **NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

## **AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

## **FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

## **UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

**ATCC Deposit No. 203331****DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

***What Is Claimed Is:***

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group  
5 consisting of:
  - (a) a polynucleotide fragment of SEQ ID NO:X or a polynucleotide fragment of the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
  - (b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:Y or a  
10 polypeptide fragment encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
  - (c) a polynucleotide encoding a polypeptide domain of SEQ ID NO:Y or a polypeptide domain encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
  - 15 (d) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:Y or a polypeptide epitope encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
  - (e) a polynucleotide encoding a polypeptide of SEQ ID NO:Y or the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X,  
20 having biological activity;
  - (f) a polynucleotide which is a variant of SEQ ID NO:X;
  - (g) a polynucleotide which is an allelic variant of SEQ ID NO:X;
  - (h) a polynucleotide which encodes a species homologue of the SEQ ID NO:Y;
  - 25 (i) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues.

2. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a secreted protein.

5 3. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:Y or the polypeptide encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X.

10 4. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:X or the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X.

15 5. The isolated nucleic acid molecule of claim 2, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

20 6. The isolated nucleic acid molecule of claim 3, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

7. A recombinant vector comprising the isolated nucleic acid molecule of claim 1.

25 8. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 1.

9. A recombinant host cell produced by the method of claim 8.

30 10. The recombinant host cell of claim 9 comprising vector sequences.

11. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

- 5 (a) a polypeptide fragment of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;
- (b) a polypeptide fragment of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z, having biological activity;
- (c) a polypeptide domain of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;
- 10 (d) a polypeptide epitope of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;
- (e) a secreted form of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;
- (f) a full length protein of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;
- 15 (g) a variant of SEQ ID NO:Y;
- (h) an allelic variant of SEQ ID NO:Y; or
- (i) a species homologue of the SEQ ID NO:Y.

20 12. The isolated polypeptide of claim 11, wherein the secreted form or the full length protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.

25 13. An isolated antibody that binds specifically to the isolated polypeptide of claim 11.

14. A recombinant host cell that expresses the isolated polypeptide of claim 11.

30 15. A method of making an isolated polypeptide comprising:  
(a) culturing the recombinant host cell of claim 14 under conditions such that said polypeptide is expressed; and

(b) recovering said polypeptide.

16. The polypeptide produced by claim 15.

5 17. A method for preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of claim 11 or the polynucleotide of claim 1.

10 18. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or absence of a mutation in the polynucleotide of claim 1; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

15 19. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or amount of expression of the polypeptide of claim 11 in a biological sample; and

20 (b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

20. A method for identifying a binding partner to the polypeptide of claim 11 comprising:

25 (a) contacting the polypeptide of claim 11 with a binding partner; and  
(b) determining whether the binding partner effects an activity of the polypeptide.

30 21. The gene corresponding to the cDNA sequence of SEQ ID NO:Y.

22. A method of identifying an activity in a biological assay, wherein the method comprises:

- (a) expressing SEQ ID NO:X in a cell;
- (b) isolating the supernatant;
- (c) detecting an activity in a biological assay; and
- (d) identifying the protein in the supernatant having the activity.

23. The product produced by the method of claim 20.

<110> Rosen et al.

<120> 31 Human Secreted Proteins

<130> PZ033

<140> Unassigned

<141> 1999-09-22

<150> 60/101,546

<151> 1998-09-23

<150> 60/102,895

<151> 1998-10-02

<160> 141

<170> PatentIn Ver. 2.0

<210> 1

<211> 733

<212> DNA

<213> Homo sapiens

<400> 1

|  |     |
|--|-----|
| gggatccgga gcccaaattct tctgacaaaa ctcacacatg cccaccgtgc ccagcacctg | 60  |
| aattcgaggg tgcaccgtca gtcttcctct tcccccaaaa acccaaggac accctcatga  | 120 |
| tctcccgagc tcctgaggtc acatgcgtgg tggaggacgt aagccacgaa gaccctgagg  | 180 |
| tcaagttcaa ctggtacgtg gacggcgtgg aggtgcataa tgccaagaca aagccgcggg  | 240 |
| aggagcagta caacagcacg taccgtgtgg tcagcgtcct caccgtcctg caccaggact  | 300 |
| ggctgaatgg caaggagtac aagtgcagg tctccaacaa agccctccca acccccatcg   | 360 |
| agaaaaccat ctccaaagcc aaagggcagc cccgagaacc acaggtgtac accctgcccc  | 420 |
| catcccgagg tgagctgacc aagaaccagg tcagcctgac ctgcctgggc aaaggcttct  | 480 |
| atccaagcga catcgccgtg gagggggaga gcaatgggca gccggagaac aactacaaga  | 540 |
| ccacgcctcc cgtgctggac tccgacggct cttcttcct ctacagcaag ctcaccgtgg   | 600 |
| acaagagcag gtggcagcag gggaaagtct tctcatgctc cgtgatgcat gaggctctgc  | 660 |
| acaaccacta cacgcagaag agcctctccc tgtctccggg taaatgagtg cgacggccgc  | 720 |
| gactctagag gat   | 733 |

<210> 2

<211> 5

<212> PRT

<213> Homo sapiens

<220>

<221> Site

<222> (3)

<223> Xaa equals any of the twenty naturally occurring L-amino acids

<400> 2

Trp Ser Xaa Trp Ser

1

5

<210> 3

<211> 86

<212> DNA



<213> Homo sapiens

<400> 3

gcgcctcgag atttccccga aatctagatt tccccgaaat gatttccccg aaatgatttc  
cccgaatat ctgccatctc aattag

60  
86

<210> 4

<211> 27

<212> DNA

<213> Homo sapiens

<400> 4

gcggcaagct ttttgcaaag cctaggc

27

<210> 5

<211> 271

<212> DNA

<213> Homo sapiens

<400> 5

ctcgagattt ccccgaaatc tagatttccc cgaaatgatt tccccgaaat gatttccccg  
aaatatctgc catctcaatt agtcagcaac catagtcccc cccctaactc cgcccatccc  
gccctaact ccgcccagtt ccgcccattc tccgcccatt ggctgactaa ttttttttat  
ttatgcagag gccgaggccg cctcggcctc tgagctattc cagaagtagt gaggaggctt  
ttttggaggc ctaggctttt gcaaaaagct t

60  
120  
180  
240  
271

<210> 6

<211> 32

<212> DNA

<213> Homo sapiens

<400> 6

gcgctcgagg gatgacagcg atagaacccc gg

32

<210> 7

<211> 31

<212> DNA

<213> Homo sapiens

<400> 7

gcgaagcttc gcgactcccc ggatccgcct c

31

<210> 8

<211> 12

<212> DNA

<213> Homo sapiens

<400> 8

ggggactttc cc

12

<210> 9

<211> 73

<212> DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 9

gcggcctcga ggggactttc ccggggactt tccggggact ttccgggact ttccatcctg  
ccatctcaat tag

60

73

&lt;210&gt; 10

&lt;211&gt; 256

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 10

ctcgagggga ctttcccggg gactttccgg ggactttccg ggactttcca tctgccatct  
caattagtca gcaaccatag tcccgcacct aactccgccc atcccgcccc taactccgcc  
cagttccgcc cattctccgc cccatggctg actaattttt tttatttatg cagaggccga  
ggccgcctcg gcctctgagc tattccagaa gtagtgagga ggcttttttg gaggcctagg  
cttttgcaaa aagctt

60

120

180

240

256

&lt;210&gt; 11

&lt;211&gt; 2007

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 11

gaattcggca cgagaacaaa acaaaacaaa acaaaaaact agatatgtaa tttctaaaca  
cccagatcac aatgacaaga tgccactcca accatgggac accttcatga tactaggttt  
gtacttcttg gtctctggga tgacttcaga ttctgtctggc caaggcaaat tgaactcagt  
tcaagatggc caccactggg agacgtgtag atagaaaaga ggactgggtct tgggaacatc  
tttgaaaaaa ccaacaaaca atagtcttag ggagatgagg aaaaaaattc accttacagt  
gctaagaaag tgcattagaa tgggaattgcc ctttccttaa ggagacagtt tgggctctcc  
ccttgccacc ggctctgggtg ttttggttta tgcgttcctt cagggttgagc tgagcagtg  
gttatgggaa gctgctcaat ttcctttcat tcaattccac ctccctctctg aactctaata  
gagggttaaaa gggaaaaaaa aaaattctgt agatagcaaa ttgtgtgtgt gggggggggt  
gggggtgtgg gtgcatggag gacaacctgc aactctgagc tccctacttc ctggctcatt  
tcatgcagtc ttttctgaac agcctatgct gctgccctgc tggcccttg tgcacggcag  
ctggccgtgt ccgtagctgt cagtatgact tagatctagc tctacctac tgggttgatgt  
gttttttctt tttgccaagt gattgagtct gtttagtagt ttccatcatt ctagtcttta  
agtaaaaatg acactattga ggaaagtcag tctactccct tcttctccc cccaaacacg  
tgttctcttt tgtcaggaaa ctgagccagt gggctgtggc agagaaagtc ctccactcag  
aggcagagac tgagttaagt cataggtggc cttaggcatc tgcattgttt gcagggggta  
agttttctt ccagtggagg ctggagggat gaattagctg gtacctgaag ccccgcttag  
ctctgacact ctgccaacat cctctgattc taggtgtggt gttgactgtc ctttcaagga  
aaaacttgca atagagggaa aagccattaa agcagctccc tgcttcatca ttaagtcctg  
tcatccctac cagccaatcc cagtcaaaga agttatgctt tattcacttc tgtggaatta  
caagtggag acacttttag gacctgatg acaaagcagg agattcactg tcagctttcc  
tggtcctctc cttacttctg tgggccttgc accgtcttag ttacacatc tgccaaaggg  
gtagaattac acttcttttt acaggtaaat gtcaaggcac aatcagtttt caggaggtgc  
ttcaagacc cagggtgaaat gaaaatgcta agtaccctct gaattggccat gcctgttacc  
aggtgctgct tcttcagatg atggggagca cttttcaggg tgaaattcag gcgagttttg  
cccaggcctg ctgtcttgag tacaatgtg gtactgcagc tgactgcttg ttgccaact  
ggaaatgttc tgtagggatt tactggcatg gtatcattcc tagaagaaaa aaagagagaa  
acttgactgc acattaaaaa aaaaaaatc cacattgtga cttttattta atttctattt  
tttttggtta taaaaagttg acttttttat ttgaatttgt cttttttatt tattggtctg  
aaaggcattt caaagggtatt ataataatat attggtgtaa tttaattggt gcaacatgct  
ttatggctcc tgtcaaaatt ggttttctc catttgattg gtttgagccc agaacagcct  
acaggggaaa aacaagctgg ataaccaccc aaagtgtttg tattttcgtt ggaaactgat  
ttttgtttca ttttggtttt tgtttctgtt tttattttta aattaaataa attgcaatga

60

120

180

240

300

360

420

480

540

600

660

720

780

840

900

960

1020

1080

1140

1200

1260

1320

1380

1440

1500

1560

1620

1680

1740

1800

1860

1920

1980

actgaaaaaa aaaaaaaaaa aactcga

2007

&lt;210&gt; 12

&lt;211&gt; 1813

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (19)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1622)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 12

|             |             |             |             |             |             |      |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| gaaatccggtt | aagctgggtnc | gcctgcaggt  | accggtccgg  | aattcccggg  | tcgacccacg  | 60   |
| cgtccgcaag  | ggcttcctgc  | ggaatgtggt  | gtcgggcgag  | cactaccgct  | ttgtgagcat  | 120  |
| gtggatggcg  | cggacgtcct  | acctggccgc  | cttcgccatc  | atggatcatc  | tcacgtgag   | 180  |
| cgtgtccatg  | ctgctgcggg  | actcacacca  | ccagatcttc  | gtcttcatcg  | cgccccctgct | 240  |
| gaccgtcatc  | ctggccctcg  | tcgggatgga  | ggccatcatg  | tcggagttct  | tcaacgacac  | 300  |
| caccaccgcc  | ttctacatca  | tcctcatcgt  | gtggtctcg   | gaccagtatg  | acgccatctg  | 360  |
| ctgccacacc  | agcaccagca  | agcggcattg  | gctgcgggttc | ttctatctct  | accacttegc  | 420  |
| cttctatgcc  | tatcactacc  | gcttcaatgg  | gcagtatagc  | agcctggccc  | tggtcacctc  | 480  |
| ctggctcttc  | atccagcatt  | ccatgatcta  | cttcttccac  | cactacgagc  | tgcttgccat  | 540  |
| cctgcagcag  | gtccgcattc  | aggagatgct  | gcttcaggcg  | ccgccactgg  | gccccgggac  | 600  |
| ccccacggcg  | ctgcccgatg  | acatgaacaa  | caactcgggc  | gccccgggcta | cagccccctga | 660  |
| ctctgccggc  | cagccccccg  | ccctggggccc | cgtctcgccy  | ggggccagcg  | ggagtcccgg  | 720  |
| gcctgtggca  | gcggcgccca  | gctccctggg  | ggccgcggca  | gcctcagtgg  | cagcagctgc  | 780  |
| cggtgggtgac | ctgggttgga  | tggcagagac  | cgctgccatc  | atcacagacg  | cctccttctc  | 840  |
| gtccggcctg  | agcgcctccc  | tcctggagcg  | gcgtccagcc  | agcccgttgg  | gccctgctgg  | 900  |
| gggcctcccc  | cacgcccccc  | aggacagtgt  | ccccccgagt  | gactccgcag  | cttctgacac  | 960  |
| aactccccctg | ggggctgcgg  | taggyggggc  | tagccccggc  | tccatggccc  | caacggaggc  | 1020 |
| gcctcgggag  | gtggggctct  | gagccgcaca  | gctgagccgc  | ctctgacccc  | tgctggctgg  | 1080 |
| gcctgacctt  | cccagagccc  | tgggggtggg  | ggaggccagc  | cacctccttc  | ctctgggact  | 1140 |
| gcccagcctg  | tgtcgggggc  | tttcagggtt  | tctggtgggt  | tgccccgaag  | gcggctttcc  | 1200 |
| tccccctggt  | gtgaggtcgc  | gcccagaggt  | tgtacccgct  | agtgaggtgt  | ttgagctggt  | 1260 |
| cagcaaggag  | aggggggtggg | gttcgcggga  | aggttcttga  | ggggctcttg  | taggtctgca  | 1320 |
| gtgaaccgtc  | ctgaggatgg  | agtgggggtc  | catggtgcag  | gtctctgagc  | aaggcggagg  | 1380 |
| tgtggaggag  | aggccggctt  | gggggtgggg  | ctcgcgccct  | agtgcgggcc  | ggcctcagcc  | 1440 |
| cggctctgcc  | tggtgctccc  | tgcagtgcct  | tctccatggc  | cccgcctccc  | ccgcgtgtgc  | 1500 |
| gccaggcctg  | gggtcccccg  | gagagcagag  | cttgccgcctc | gggcataggg  | acgtgggggtg | 1560 |
| caggcgccaa  | catcagtggc  | agcagccagg  | gccgtgggtc  | agtcccactc  | ggggatggag  | 1620 |
| tnggccggcg  | gccaaaccag  | tcactcgggg  | aggaatgcgg  | aggagcgctc  | attccattct  | 1680 |
| atttaattgc  | agtgtacaaa  | attgtgtttg  | tatatagaat  | aaactgtctg  | ttgacagcga  | 1740 |
| aaaaaaaaaa  | aaaaaaaaaa  | aaaaaagggc  | ggccgctcta  | gaggatccaa  | gcttacgtac  | 1800 |
| gcgtgcatgc  | gac         |             |             |             |             | 1813 |

&lt;210&gt; 13

&lt;211&gt; 2808

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 13

tcgacccacg cgtccgagcg tgcccaccag ccatggcggg gccccggggc ctctctccac

60

|             |             |            |             |             |             |      |
|-------------|-------------|------------|-------------|-------------|-------------|------|
| tctgcctcct  | ggccttctgc  | ctggcagget | tcagcttctg  | cagggggcag  | gtgctgttca  | 120  |
| aaggctgtga  | tgtgaaaacc  | acgtttgtca | ctcatgtacc  | ctgcacctcg  | tgcgcggcca  | 180  |
| tcaagaagca  | gacgtgtccc  | tcaggctggc | tgcgggagct  | cccggatcag  | ataaaccagg  | 240  |
| actgcccgtg  | cggggccacc  | ctgtccttgc | ctgtgtccag  | gagcatcctg  | tgggggtggca | 300  |
| gggactcggg  | gagcctgaca  | ggcccaaaaa | atgaggagaa  | gcactcactt  | atccatgccc  | 360  |
| ctgtggcccc  | acctgggtgg  | tggagatagg | cggggaggaa  | ggggtaaagg  | cgaggggcag  | 420  |
| gaggggttta  | tggggcacct  | gctatgtgct | aggccctgtg  | ctcagctctg  | ggccaggcag  | 480  |
| agcagggagg  | tgggtgggag  | ccccggaagc | caatgtgggg  | aggctgggtg  | ggggtgtgtc  | 540  |
| aaggggagcct | tttccatcac  | cctgcccacc | ctctgcccc   | agctacgaat  | acagctgggg  | 600  |
| ggctctatgg  | tgtccatgag  | cggctgcaga | cggagtgcc   | ggaagcaagt  | ggtgcagaag  | 660  |
| gcctgtgtcc  | ctggctactg  | gggttcccgg | tgccatgaat  | gccctggggg  | cgctgagacc  | 720  |
| ccatgcaatg  | gccacgggac  | ctgcttggat | ggcatggaca  | ggaatgggac  | ctgtgtgtgc  | 780  |
| caggaaaaact | tccgcggctc  | agcctgccag | gagtgccaa   | acccaaccg   | gttcggggcct | 840  |
| gactgccaat  | cgggtgtgcag | ctgtgtgcac | ggagtgtgca  | accatggggc  | acgtggggat  | 900  |
| ggaagctgcc  | tgtgtctttg  | tggatacact | ggccccact   | gtgatcaaga  | gctgcccgtc  | 960  |
| tgccaggagc  | tgcgctgtcc  | ccagaacacc | cagtgtctcg  | cagaggctcc  | cagctgcagg  | 1020 |
| tgcctgcccc  | gctacacaca  | gcagggcagt | gaatgccgag  | cccccaaccc  | ctgctggcca  | 1080 |
| tcacctgtct  | cactgctggc  | ccagtgtctg | gtgagcccca  | aggggcaggc  | tcagtgtcac  | 1140 |
| tgccttgaga  | actaccatgg  | cgatgggatg | gtgtgtctgc  | ccaaggaccc  | atgcactgac  | 1200 |
| aaccttgggtg | gctgccccag  | caactctact | ttgtgtgtgt  | accagaagcc  | gggccaggcc  | 1260 |
| ttctgcacct  | gccggccagg  | cctggtcagc | atcaacagca  | acgcttctgc  | gggtgtcttc  | 1320 |
| gccttctgct  | cccccttctc  | ctgogaccgg | tctgccactt  | gccagggtgac | cgctgatggg  | 1380 |
| aagaccagct  | gtgtgtgcag  | ggaagcgagg | tgggggatgg  | gcgtgcctgc  | tacggacacc  | 1440 |
| tgtctcacga  | ggtgcagaag  | gccacgcaga | caggccgggt  | gttctctgcag | ctgagggtcg  | 1500 |
| ccgtggccat  | gatggaccag  | ggctgccggg | aaatccttac  | cacagcgggc  | cctttcacccg | 1560 |
| tgctggtgcc  | atccgtctcc  | tccttctcct | ccaggacctt  | gaatgcaccc  | cttgcccagc  | 1620 |
| agctctgtag  | acagcacatc  | atcgcagggc | agcacatcct  | ggaggacaca  | aggacccaac  | 1680 |
| aaacacgaag  | gtggtggacg  | ctggccgggc | aggagatcac  | cgtcaccttt  | aaccaattca  | 1740 |
| cgaataactc  | ctacaagtac  | aaagaccagc | cccagcagac  | gttcaacatc  | tacaaggcca  | 1800 |
| acaacatagc  | agctaattgg  | gtcttccacg | tggctactgg  | ctgcggtggg  | caggccccct  | 1860 |
| ctgggacccc  | tggggatccc  | aagagaacta | tcggacagat  | cctcgccctc  | accgaggcct  | 1920 |
| tcagccgctt  | tgaaccatc   | ctggagaact | gtgggctgcc  | ctccatcctg  | gacggacctg  | 1980 |
| ggcccttcac  | agtcttttgc  | ccaagcaatg | aggctgtgga  | cagcttgctg  | gacggccgcc  | 2040 |
| tgatctacct  | cttcacagcg  | ggtctctcta | aactgcagga  | gttggtgcgg  | taccacatct  | 2100 |
| acaaccacgg  | ccagctgacc  | ggtgagaagc | tcatctccaa  | gggtcggatc  | ctcaccatgg  | 2160 |
| cgaaccaggt  | cctggctgtg  | aacatttctg | aggaggggcg  | catectgtctg | ggacccgagg  | 2220 |
| gggtcccgtc  | gcagagggtg  | gacgtgatgg | ccgccaatgg  | tgtgatccac  | atgctggacg  | 2280 |
| gcatectgct  | gccccgacc   | atcctgcccc | tctgcccac   | gactgcagc   | gaggagcagc  | 2340 |
| acaagattgt  | ggcgggctcc  | tgtgtggact | gccaagccct  | gaacaccagc  | acgtgtcccc  | 2400 |
| ccaacagtgt  | gaagctggac  | atcttcccc  | aggagtgtgt  | ctacatccat  | gacccaacgg  | 2460 |
| ggctcaatgt  | gctaaagaag  | ggctgtgcca | gctactgcaa  | ccaaaccatc  | atggaacaag  | 2520 |
| gctgtgtcaa  | aggttttttc  | gggctgtact | gcacgcagtg  | tcctgggggc  | ttctccaacc  | 2580 |
| cctgtctatg  | caaaggcaat  | gtgagtccca | tcctctctctg | gggtgaggta  | tggggaacac  | 2640 |
| aaggactcct  | tcaccgggtg  | gccagtgaat | ggctgtgtgt  | ctgggccaag  | cctgccactc  | 2700 |
| tggccttggg  | attctcatat  | ctatgcagtg | gaaagtgtga  | tcaaattatt  | tctcatattt  | 2760 |
| taattaaaaa  | taattagaga  | gaaaaaaaaa | aaaaaaaaaa  | aaaaaaaaa   |             | 2808 |

&lt;210&gt; 14

&lt;211&gt; 1389

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 14

|            |            |            |            |            |            |     |
|------------|------------|------------|------------|------------|------------|-----|
| gaaaaaggca | gagtctgtgt | catgattgga | gtccagtgtt | gcacttttat | ccctattaac | 60  |
| acagccccta | atggaacaat | tacaaaagca | ttacagggcc | ttacaagtga | attagctgaa | 120 |
| aatcccggaa | taaatggccc | cttttcaggt | cttatggaaa | aatgggtttg | aaaatggaaa | 180 |
| tgactcatga | cctcaatatt | tacttctctt | gcagttgtta | caggtgtact | cattcttgta | 240 |
| ggttgctgta | ttacaccttc | tgttcatggg | ttggtgcaaa | ggcttacaga | aacagctctt | 300 |

|             |             |            |            |            |             |      |
|-------------|-------------|------------|------------|------------|-------------|------|
| actaaaacct  | ccctcaattc  | ttccccaccc | tattcagata | aactcccact | tctagatcac  | 360  |
| caagaggaac  | aacagagcca  | aattatgttt | gaaaaatttg | aagagggaaa | actataaaga  | 420  |
| gggggatatt  | gccaggaacc  | ataagttctc | ttcaaacttc | gtggtccttc | tgggtctgta  | 480  |
| aacaaccctt  | cccacctaatt | tggtaccagg | accagcctct | tccttcctgc | ctaataaggcc | 540  |
| ctattcaatt  | tcaaacaata  | gccaatcggg | tttagatggt | gtggccaac  | tctagttact  | 600  |
| ggggaaagga  | cacagaaaca  | tgagctgcat | taggggtaaa | aaccccctgc | cctagccccc  | 660  |
| ttgggtgtgct | cttgcaattg  | ctttcagatg | caagtagcac | cctttctgca | gaagtaaagt  | 720  |
| tgtcttgctg  | aggaattttc  | agtctaagtg | ctagttcttt | ctttgtggca | cccaacacct  | 780  |
| gttcccaaca  | atgagaatat  | caacaaatag | gaaatataac | aaagaaccaa | acagaaatga  | 840  |
| agataaagat  | acaataatga  | aaattaaaaa | ttcactggaa | gtgtttgaca | tattatttga  | 900  |
| ttccattctt  | cctaaatatg  | atttcaatct | tcttaaattg | attcagtcct | gtttgtgac   | 960  |
| ctatcctgga  | aatatgtgat  | gtgatctatc | ctggagaatg | ttctgtgtgc | acttgagaag  | 1020 |
| attacatatt  | tttcttttgt  | taattgaaaa | attctgtata | tggtatttac | atcaatttaa  | 1080 |
| agtccactat  | tttgttattg  | attttctatc | tggattttct | attttttatt | gaaagtgggg  | 1140 |
| tactgaatta  | ttctaccatt  | acactattgc | tgtccatttc | tcctttcaga | tattttgata  | 1200 |
| tttgttctat  | atacttaggt  | agtctaatag | taggtacata | tgtatttaca | attgctgtta  | 1260 |
| tcttcctgtt  | gaattcaccc  | ttttatcatt | atgtaatgac | tttgtctcct | gggacagttt  | 1320 |
| ttgacttaaa  | ttctaatttg  | tgctatataa | gaaaaaaaaa | aaaaaaaaaa | aaaaaaaaaa  | 1380 |
| aaaaaaaaa   |             |            |            |            |             | 1389 |

&lt;210&gt; 15

&lt;211&gt; 1873

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 15

|             |             |             |             |             |             |      |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| ctccctgcaa  | ctaagttttt  | cctgggggaaa | aagcctgcag  | accaggaagt  | gggaggtgtc  | 60   |
| tggtgggaca  | ctgccctcaa  | aaggagcctt  | tctgagtgtc  | ggggcttcat  | ctgccagggc  | 120  |
| tgcacctcag  | caggcagtg   | gctcccagag  | ctggctgcag  | taaccgcag   | gctgtgccgg  | 180  |
| caggatgtgg  | tctttgggtt  | ctgtttctgt  | tttagttctg  | acttgtgccg  | tggtgtgggc  | 240  |
| tgagggccta  | gggtggggag  | aagtctccac  | aggtgggatt  | gaactcccca  | ggcacatggt  | 300  |
| ccttgtgggtg | ctgggtggaga | gagagttccc  | agaggtttcg  | gacatgctac  | cgtaaaaacc  | 360  |
| ttttcctcaa  | ggtgataggt  | acgtgtcaag  | gtgaccctg   | tccgccatcc  | ggcagcttgg  | 420  |
| aagaagccgg  | taagatggac  | agatcagggg  | gggttggagg  | gtcccgtttg  | tgaaggagct  | 480  |
| ggggaggggg  | aggcaaggct  | gagcacagca  | gcccactgtg  | atgctctcag  | tgcagaggcc  | 540  |
| ccgggggggag | acccaggggg  | gcacccccca  | aaaccacacc  | atacagttag  | tttcagttcc  | 600  |
| cccaactctg  | cccttttca   | tctcctcggc  | tgccctggctg | cctcacaggt  | tgtcagcttg  | 660  |
| agcaaggggc  | caagcggcct  | ctactcagag  | accctgggtg  | tcagagggga  | gtgggggaagg | 720  |
| tggacatggg  | catcagcaca  | gaccagcacc  | tctgggtccat | cctgcctggt  | catccacagg  | 780  |
| ggagtgccag  | ctcccacccc  | cagccacata  | ctgttctcag  | cttctgagtg  | gctgggctgt  | 840  |
| cccagagggc  | catgctgggc  | ctgagtgtctg | tccttggggca | tatgcagatg  | gtgttgtatc  | 900  |
| caggggggtt  | tcagggaagc  | tttagcactt  | tcatattaaa  | acaaaaaatg  | ttggaatctc  | 960  |
| cattaaatag  | tcaagtctta  | gggatagtag  | taaaaacatg  | gctgtacatg  | gctggccagg  | 1020 |
| cacagtggct  | cacgcctata  | atcctagcac  | tttggggaggc | tgaggtggga  | gggttggcca  | 1080 |
| agagcttgag  | accagcctgg  | tcaacatagc  | aagaccgctc  | tctacttgat  | tttttttttt  | 1140 |
| aaagtacatg  | gcaaagggca  | ttataaagta  | agatttgtat  | tagctttag   | ctggttactg  | 1200 |
| atgttcatag  | gaagggcctc  | aaccataata  | atgggtgaaat | aataaaatga  | gatgaataaa  | 1260 |
| tattagagag  | gtagaatagt  | acgtagaaag  | gttttagcaca | cattttgttt  | ttctgcacga  | 1320 |
| ggtggttgtt  | ctctgctttc  | aaagtgtctga | aacgaatgtc  | ctacattgtc  | tgccttccat  | 1380 |
| gaggttctga  | ccgcccttga  | gaacagatgt  | accactaca   | gggtgaaggt  | gagaatgtaa  | 1440 |
| tttgaagctg  | aatgagaatg  | gagctggatt  | ctaaaattat  | atccccctctc | agattcacia  | 1500 |
| gtgttcacac  | atttaggcaa  | gaagccctaa  | gtcctaacc   | tggacatgtg  | gattgtccag  | 1560 |
| gaccatccca  | gacttttgtg  | gctggattaa  | tcagacctga  | gcaaactgga  | tgcaaagagg  | 1620 |
| agggggtagg  | ttccgggaag  | gggccctggt  | ggccaggggtg | ggatgagtg   | ctgggtgctga | 1680 |
| gcccagagcc  | caggcccttg  | caatgttttt  | cttggatctg  | ctgggggcag  | tttaagctaa  | 1740 |
| taaaagcctc  | ctcttttatt  | cccagggaca  | catggaagct  | gcctcgtgcc  | gaattcgata  | 1800 |
| tcaagcttat  | cgataccgtc  | gacctcgagg  | gggggcccgg  | taccaatttc  | gccctatagt  | 1860 |
| gagtcgtatt  | aca         |             |             |             |             | 1873 |

<210> 16  
 <211> 2409  
 <212> DNA  
 <213> Homo sapiens

<400> 16

|             |            |             |             |             |             |      |
|-------------|------------|-------------|-------------|-------------|-------------|------|
| cgccacgagc  | ggctcccggc | gccatgtgag  | ggggctcggg  | ggccgcgggg  | ggccgggggc  | 60   |
| tccccgccgg  | agatgaggaa | actgaggctc  | agagagggtc  | catggcttgc  | tgcaggcccc  | 120  |
| ccagccagtg  | tgaaccacac | tccctgcccc  | cagggccacc  | tgaggagcgc  | cgacacctac  | 180  |
| ccctcagcag  | acgccggaga | gaaatgagta  | gcaacaaaga  | gcagcgggtc  | gcagtggtcg  | 240  |
| tgatcctctt  | tgccctcatc | accatcctca  | tcctctacag  | ctccaacagt  | gccaatgagg  | 300  |
| tcttccatta  | cggctccctg | cggggccgta  | gccgccgacc  | tgtcaacctc  | aagaagtgga  | 360  |
| gcatcactga  | cggctatgtc | cccattctcg  | gcaacaagac  | actgccctct  | cggtgccacc  | 420  |
| agtgtgtgat  | tgtagcagc  | tccagccacc  | tgctggggcac | caagctgggc  | cctgagatcg  | 480  |
| agcgggctga  | gtgtacaatc | cgcagtgaatg | atgcacccac  | caactggctac | tcagctgatg  | 540  |
| tggggcaaca  | gaccacctac | cgcgtcgtgg  | cccattccag  | tgtgttccgc  | gtgctgagga  | 600  |
| ggccccagga  | gtttgtcaac | cggacccctg  | aaaccgtgtt  | catcttctgg  | gggccccga   | 660  |
| gcaagatgca  | gaagccccag | ggcagcctcg  | tgctgtgtgat | ccagcgagcg  | ggcctggtgt  | 720  |
| tccccaacat  | ggaagcatat | gccgtctctc  | ccggccgcac  | gaggcaattt  | gacgacctct  | 780  |
| tccggggtga  | gacgggcaag | gacagggaga  | agtctcatte  | gtgggttgagc | acaggctggt  | 840  |
| ttaccatggt  | gatcgcggtg | gagttgtgtg  | accacgtgca  | tgtctatggc  | atgggtcccc  | 900  |
| ccaactactg  | cagccagcgg | ccccgcctcc  | agcgcagtc   | ctaccactac  | tacgagccca  | 960  |
| aggggcccga  | cgaatgtgtc | acctacatcc  | agaatgagca  | cagtcgcaag  | ggcaaccacc  | 1020 |
| accgcttcat  | ccgagaaaag | ggtcttctca  | tcgtggggccc | agctgtatgg  | catcaccttc  | 1080 |
| tcccaccctc  | cctggaccta | ggccaccag   | cctgtgggac  | ctcaggaggg  | tcagaggaga  | 1140 |
| agcagcctcc  | gcccagccgc | taggccaggg  | accatcttct  | ggccaatcaa  | ggcttgcttg  | 1200 |
| agtgtctccc  | agccaatcag | ggccttgagg  | aggatgtatc  | ctccagccaa  | tcagggccctg | 1260 |
| gggaatctgt  | tggcgaatca | gggatttggg  | agtctatgtg  | gttaatcagg  | ggtgtctttc  | 1320 |
| ttgtgcagtc  | aggggtctcg | cacagtcaat  | cagggtagag  | ggggtatttc  | tgagtcaatc  | 1380 |
| tgaggctaag  | gacatgtcct | ttcccatgag  | gccttggttc  | agagccccag  | gaatggaccc  | 1440 |
| cccaactact  | ccccactctg | ctgggataat  | gggggtcctgt | cccaaggagc  | tgggaacttg  | 1500 |
| gtgttgcccc  | ctcaatttcc | agcaccagaa  | agagagattg  | tgtgggggta  | gaagctgtct  | 1560 |
| ggaggcccgg  | ccagagaatt | tgtgggggtg  | tggagggttg  | gggggcgggtg | gggagggtccc | 1620 |
| agagggtggga | ggctggcatc | caggtcttgg  | ctctgccctg  | agaccttgga  | caaacccttc  | 1680 |
| ccctctcttg  | ggcacccttc | tgcccacacc  | agtttccagt  | gcggagtctg  | agaccctttc  | 1740 |
| cacctcccct  | acaagtgcct | tcgggtctgt  | cctccccgtc  | tggaccctcc  | cagccactat  | 1800 |
| cccttgctgg  | aaggctcagc | tctttggggg  | gtctgggggtg | acctccccac  | ctcctggaaa  | 1860 |
| acttttaggt  | acttttgctc | aaactccttc  | aggggtgggg  | gactctgaag  | gaaacgggac  | 1920 |
| aaaaccttaa  | gctgttttct | tagccctca   | gccagctgcc  | attagcttgg  | ctcttaaagg  | 1980 |
| gccaggcctc  | cttttctgcc | ctctagcagg  | gaggttttcc  | aactgttgga  | ggcgcccttg  | 2040 |
| gggctgcccc  | tttgtctgga | gtcactgggg  | gcttccgagg  | gtctccctcg  | acctctgtc   | 2100 |
| gtcctgggat  | ggctgtcggg | agctgtatca  | cctgggttct  | gtccccctggc | tctgtatcag  | 2160 |
| gcactttatt  | aaagctgggc | ctcagtgggg  | tgtgtttgtc  | tcctgctctt  | ctggagcctg  | 2220 |
| gaaggaaagg  | gcttcaggag | gaggctgtga  | ggctggaggg  | accagatgga  | ggaggccagc  | 2280 |
| agctagccat  | tgacactggg | ggtgatgggt  | gggggcgggtg | actgccccag  | acttggtttt  | 2340 |
| gtaatgattt  | gtacaggaat | aaacacacct  | acgtcccgaa  | aaaaaaaaaa  | aaaaaaaaaa  | 2400 |
| aaaaaaaaaa  |            |             |             |             |             | 2409 |

<210> 17  
 <211> 1590  
 <212> DNA  
 <213> Homo sapiens

<400> 17

|             |            |            |            |            |             |     |
|-------------|------------|------------|------------|------------|-------------|-----|
| cccacgcgtc  | cgagcggcgt | tgccatggcg | gcgtctcttg | ggcaggtgtt | ggctctgggtg | 60  |
| ctgggtggccg | ctctgtgggg | tggcacgcag | ccgctgctga | agcgggcctc | cgccggcctg  | 120 |

|             |             |             |             |             |            |      |
|-------------|-------------|-------------|-------------|-------------|------------|------|
| cagcggggttc | atgagccgac  | ctgggcccag  | cagttgctac  | aggagatgaa  | gaccctcttc | 180  |
| ttgaatactg  | agtacctgat  | gccctttctc  | ctcaaccagt  | gtggatccct  | tctctattac | 240  |
| ctcaccttgg  | catcgacagg  | ctgggtcccag | acctcagagt  | tcagggtcttc | atgctggaat | 300  |
| cctgggaagc  | actgaacgtg  | gagtcaggag  | atgtgggttt  | gagtcctgag  | atttgctgtg | 360  |
| acttcatctg  | gaagatgggg  | ttttgatacc  | ttttttaaag  | atctgaccct  | ggctgtgccc | 420  |
| atctgtaact  | ctctggctat  | catcttcaca  | ctgattgttg  | ggaaggccct  | tggagaagat | 480  |
| attgggtgaa  | aacgtaagtt  | agactactgc  | gagtgccgga  | cgcagctctg  | tggatctcga | 540  |
| catacctgtg  | ttagttcctt  | cccagaacct  | atctccccag  | agtgggtgag  | gacacggcct | 600  |
| tttcccatcc  | tgccttttcc  | tctgcagctg  | ttttgcttcc  | ttgtggccat  | cagagttccc | 660  |
| ttcccctgga  | cagtcctggag | aaagacagag  | ctgggggtttg | ggattgaaga  | ccagacccca | 720  |
| tctgagccct  | tccctccagcc | ctgtaccagc  | tctactggc   | atggctgagc  | tcagaccctc | 780  |
| ctgattttctg | cctattatcc  | caggagcagt  | tgctggcatg  | gtgctcaccg  | tgataggaat | 840  |
| ttcactctgc  | atcacaagct  | cagtgaagta  | gaccagggg   | caacagtcta  | ccctttgagt | 900  |
| gggcccgaacc | cacttccagc  | tctgctgcct  | ccaggaagcc  | cctgggccc   | gaagtgtctg | 960  |
| cagtgaagcgg | gatgggacct  | agcacttccc  | ctctctggcc  | ttagcttctc  | cctctcttat | 1020 |
| ggggataaca  | gctacctcat  | ggatcacaa   | aagagaacaa  | gagtgaagaa  | gttttgtaac | 1080 |
| cttcaagtgc  | tggtcagctg  | cggggattta  | gcacaggaga  | ctctacgctc  | accctcagca | 1140 |
| acctttctgc  | cccagcagct  | ctcttctctg  | taacatctca  | ggctcccagc  | ccagccacca | 1200 |
| ttactgtggc  | ctgatctgga  | ctatcatggg  | ggcaggttcc  | atggactgca  | gaactccagc | 1260 |
| tgcatggaaa  | gggccagctg  | cagactttga  | gccagaaatg  | caaacgggag  | gcctctggga | 1320 |
| ctcagtcaga  | gcgctttggc  | tgaatgaggg  | gtggaaccga  | gggaagaagg  | tgctctggga | 1380 |
| tgccagatgc  | aggaaatgag  | ctgtctatta  | gccttgccctg | ccccacccat  | gaggtaggca | 1440 |
| gaaatcctca  | ctgccagccc  | ctcttaaaaca | ggtagagagc  | tgtgagcccc  | agccccacct | 1500 |
| gactccagca  | cacctggcga  | gtagtagctg  | tcaataaate  | tatggtaaac  | agaaaaaaaa | 1560 |
| aaaaaaaaaa  | aaaaaaaaaa  | aaaaaaaaaa  |             |             |            | 1590 |

&lt;210&gt; 18

&lt;211&gt; 1567

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (36)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 18

|             |             |             |            |             |            |      |
|-------------|-------------|-------------|------------|-------------|------------|------|
| acacgggagc  | ggcgcgccc   | ggtcgaccca  | cgcgtncggg | ycgggygccg  | ctaactggag | 60   |
| cgaaccccag  | cgtscgcga   | catggcctgg  | accaagtacc | agctgttcct  | ggccgggtc  | 120  |
| atgcttggtta | ccggctccat  | caacacgctc  | tgggcaaaat | gggcgggaca  | tttcatggcc | 180  |
| gagggctgtg  | gagggagcaa  | ggagcacagc  | ttccagcatc | ccttccctca  | ggcagtgggc | 240  |
| atgttccttg  | gagaattctc  | ctgcctggct  | gccttctacc | tccctccgatg | cagagctgca | 300  |
| gggcaatcag  | actccagcgt  | aracccccag  | cagcccttca | accctcttct  | tttctgccc  | 360  |
| ccagcgctct  | gtgacatgac  | agggaccagc  | ctcatgtatg | tggctctkaa  | catgaccagt | 420  |
| gcctccagct  | tccagatgct  | gcgggggtgca | agtgatcata | ttcactggcc  | tggtctcggt | 480  |
| ggccttccctg | ggccgggargc | tggtgctgag  | ccagtggctg | ggcatcctag  | ccaccatcgc | 540  |
| ggggctgggtg | gycgtgggccc | tggtkacct   | cctgagcaag | cacgacagtc  | aagcacaagc | 600  |
| ttcagcgaag  | tgatcacagg  | ggacctgttg  | atcatcatgg | ccagatcat   | cgttgccatc | 660  |
| cagatgggtgc | tagaggagaa  | gttcgtctac  | aaacacaatg | tgcaccact   | gcgggcagtt | 720  |
| ggcactgagg  | gcctcttttg  | ctttgtrate  | ctctccctgc | tgctggtgcc  | catgtactac | 780  |
| atccccgcgc  | gctccttcag  | cggaaaccct  | cgtgggacac | tggaggatgc  | attggacgcc | 840  |
| ttctgccagg  | tgggccagca  | gccgctcatt  | gccgtggcac | tgctgggcaa  | catcagcagc | 900  |
| attgccttct  | tcaacttcgc  | aggcatcagc  | gtcaccaagg | aactgagcgc  | caccacccgc | 960  |
| atggtgttgg  | acagcttgcg  | caccgtttgc  | atctgggcac | tgagcctggc  | actgggctgg | 1020 |
| ragsccttcc  | atgcactgca  | gatccttggc  | ttcctcatac | tccttatagg  | cactgcctc  | 1080 |
| tacaatgggc  | tacaccgtcc  | gctgctgggc  | cgmetgtcca | ggggccggcc  | cctggcagag | 1140 |
| gagagcgagc  | aggagagact  | gctgggtggc  | accgcactc  | ccatcaatga  | tgccagctga | 1200 |
| ggttcctctg  | aggcttctac  | tgccacccgg  | gtgctccwte | tmmctgagac  | tgaggccaca | 1260 |

|            |            |            |            |            |            |      |
|------------|------------|------------|------------|------------|------------|------|
| caggctggtg | ggccccgaat | gccctatccc | caaggcctca | cctgtcccc  | tccctgcaga | 1320 |
| acccccaggg | cagctgctgc | cacagaagat | aacaacaccc | aagtctctt  | tttctcacta | 1380 |
| cacctgcagg | gtggtgttac | ccagccccca | caagcctgag | tgcagtggca | gacctcagct | 1440 |
| ctctggaccc | ctcctacagc | actagagcta | aatcatgaag | ttgaattgta | ggaatttacc | 1500 |
| accgtagtgt | atctgaatca | taaactagat | tatcataaaa | aaaaaaaaaa | aaaaaaaggg | 1560 |
| cgggcgc    |            |            |            |            |            | 1567 |

&lt;210&gt; 19

&lt;211&gt; 3430

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 19

|             |             |             |             |             |             |      |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| cagacatgca  | gaaagtacga  | atctgctctg  | agggcgggatg | gaaaatgaag  | aatctgacgt  | 60   |
| aaagcctcca  | gactggccaa  | acccaatgaa  | tgctacctcc  | cagtttcctc  | agcctcagca  | 120  |
| ctttgacagc  | tttggcctcc  | gtctgcctcg  | ggatatcaca  | gagctgcccg  | agtggagtga  | 180  |
| ggggtaaccc  | ttctacatgg  | ccatgggctt  | cccaggggat  | gacctctcgg  | ctgatgacat  | 240  |
| agctgggaag  | tttcagttca  | gccggggcat  | gcgcccgcagt | tacgacgcag  | ggttcaagct  | 300  |
| gatggtagt   | gaatatgctg  | agagtaccaa  | caactgccag  | gctgccaaagc | agtttggagt  | 360  |
| attggaaaaa  | aacgttccgag | actggcgcaa  | agtgaagcca  | cagcttcaaa  | acgcccacgc  | 420  |
| catgcccggg  | gcattccgag  | gccccaaaga  | tgggaggttt  | gctctggtgg  | accagcgtgt  | 480  |
| ggccgaatat  | gtcagataca  | tgacggccaa  | aggggacccc  | atcacccggg  | aggcgatgca  | 540  |
| gctgaaagct  | ctcgaaatcg  | cccaggaaat  | gaacattcca  | gagaaagggg  | tcaaggcaag  | 600  |
| cttgggttgg  | tgctgaagaa  | tgatgagaag  | gtatgacctg  | tctctgaggc  | ataaagtgcc  | 660  |
| cgtgccccag  | cacctgccgg  | aagacctgac  | tgagaaactc  | gtcacttacc  | agcgagtggt  | 720  |
| cctggctctg  | cgcagggcgc  | atgactatga  | ggtagctcag  | atgggggaatg | cagatgagac  | 780  |
| gcccatttgt  | ttagaggtgc  | catcacgggt  | aactgttgat  | aaccagggcg  | aaaagcctgt  | 840  |
| cttgggtcaag | acaccaggca  | gggaaaaact  | gaaaatcaca  | gcaatgcttg  | gtgtcttggc  | 900  |
| tgatgggagg  | aagttaccac  | cgtacatcat  | tttgagggga  | acatatatcc  | ccccggggaa  | 960  |
| gtttcccaag  | gggatggaaa  | ttcgctgccca | ccggtatggg  | tggatgactg  | aagacttgat  | 1020 |
| gcaggactgg  | ttggaagtgg  | tgtggagacg  | gaggacagga  | gcagtgccca  | agcagcgagg  | 1080 |
| gatgctgac   | ttgaatggct  | tccggggcca  | tgccacagat  | tccgtgaaga  | actccatgga  | 1140 |
| aagcatgaac  | actgacatgg  | tgatcatccc  | aggggggtctg | acctcacagc  | ttcaggtgct  | 1200 |
| ggatgtcgtg  | gtctacaagc  | cactgaatga  | cagtgtgcgg  | gcccagttact | ccaactggct  | 1260 |
| tctggctggg  | aacctggcgc  | tgagcccaac  | cgggaaatgct | aagaagccac  | ccctgggcct  | 1320 |
| ctttctggag  | tgggtcatgg  | tcgcgtggaa  | tagcatctca  | agtgagtcca  | tcgtccaagg  | 1380 |
| gttcaagaat  | tgccatatct  | ccagcaactt  | ggaggaggaa  | gacgatgtcc  | tgtgggaaat  | 1440 |
| cgagagtgg   | ttgccaggag  | gaggagaacc  | accaaagat   | tgtgacaccg  | aaagcatggc  | 1500 |
| tgagagcaac  | tgaagggaag  | gggaaagcaa  | atggaaactct | gatttaaaca  | gctggggatg  | 1560 |
| aaattcctca  | agatgattat  | tcctgaaagt  | gtggatgcgc  | tggatgcgca  | gggaacatca  | 1620 |
| ggaaaaggcc  | acggggctct  | gaacagcccc  | ggtccagaca  | gcagcctgta  | catccatccc  | 1680 |
| aggacacagc  | ccagcccctc  | cccacaccat  | acaaggatct  | agaaaagtct  | aggacctacc  | 1740 |
| atttcatcag  | agacatgac   | agaaaagaaa  | ctgcttctgc  | cccatttctt  | gttttggaga  | 1800 |
| ttactccatc  | tgtccatcaa  | aagaaacctg  | taaatatgaa  | agaacaaagg  | ttatttcctg  | 1860 |
| gagaaaagac  | aatttattca  | acaccaacaa  | gggactcatc  | atatgggcac  | aactctgggtg | 1920 |
| tccttctatg  | gagaaaacct  | caagtaaagt  | tttattctgc  | ctttgaaaat  | gcttccaaaa  | 1980 |
| gtagaccctg  | tccccacaca  | ggtcaagact  | acagagaagg  | ctttgtagaa  | atgtgtcacc  | 2040 |
| tatgtacacc  | tgctacttac  | acatttcctc  | ttttggaaaa  | atgagatact  | tagaataaca  | 2100 |
| agaaaattaa  | gacatactgg  | cctggtgcca  | gcagatggct  | tttctataga  | caaactaggt  | 2160 |
| tagtgtggaa  | gatatagggt  | aaaataaact  | atgctgtttt  | atztatcttc  | ccaacctgat  | 2220 |
| tggcagctag  | acttttttag  | ggtctcattt  | aatggccctg  | tttttttcat  | tattatatatt | 2280 |
| aatgataggg  | caggatttcg  | tatgcaagct  | cttgtttctc  | aggetgcctg  | cagaagaagt  | 2340 |
| cgctataaat  | tatctgttgt  | ctacatggta  | caaggcccat  | tgactcatct  | gatgcttggt  | 2400 |
| ttgttaattt  | cttaatatatt | ttatcacggg  | gcagtgggag  | ggcttgggct  | tttagccaca  | 2460 |
| gctgttttaa  | gacttctgat  | ctcctgccct  | gcaggaatag  | gtgggaagtc  | attgaatttt  | 2520 |
| tacactatag  | taatttgcac  | tcccacataa  | gtttgagtgt  | tacgaaaaca  | ttcctttaaa  | 2580 |
| gggatctgtg  | ctacacaaaa  | tatgccagga  | cctcacagac  | aaagccattg  | ctagaaatgt  | 2640 |
| cattccaatg  | atcagatctg  | gaaacaggct  | gccataacca  | cttttctctc  | ttgtagactc  | 2700 |



|            |            |            |            |            |            |      |
|------------|------------|------------|------------|------------|------------|------|
| agctcacctg | tatattttaa | ctgttcttgg | catcttgaaa | cacctatttc | tactcaggta | 2760 |
| ctcattgtcc | tgttactgat | tcacctttct | gacccctttc | aaccagtttt | cccccaagg  | 2820 |
| gggaaatttt | acttaacctc | tagtatttga | acaactcaat | atttgaattg | ttgccccatt | 2880 |
| tgcttttacc | tgtactgtat | tcttggtcat | ctcaaattgg | gtctaaaccc | agctactttg | 2940 |
| cattccagaa | gtttccattc | cctccaattc | cacctaat   | ttcatctgtc | ctagttactg | 3000 |
| gctctttctt | catgtcttat | ttctcttggc | ttgggagctt | aaaagatttt | acaagacctc | 3060 |
| atcttgggtt | ccttccttgg | agccatagtt | accctgccaa | gaagagtaga | aaatgggttc | 3120 |
| aactcctgtt | tcgctccacc | aacacctctg | tgagtctcat | catcagctga | gcgatgatgc | 3180 |
| cttacagggt | gcatagcact | ggaactttcc | tagagtaacg | gctctgctgc | cagggtttct | 3240 |
| ctgggctcat | tcttccactg | acttaattat | gatctatgcc | taacagagcc | ccagtacaac | 3300 |
| tattttgcag | aatggctgtt | accctagaat | tactatagca | catattgaga | tatagttgta | 3360 |
| ctccctagta | gataggaact | gacccaaca  | ataaactttg | ataataaaaa | aaaaaaaaaa | 3420 |
| aaaaaaaaaa |            |            |            |            |            | 3430 |

&lt;210&gt; 20

&lt;211&gt; 1529

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1505)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 20

|            |             |            |             |             |            |      |
|------------|-------------|------------|-------------|-------------|------------|------|
| gcggttgccg | ccgccgccga  | tcagctgagc | wgagacggag  | ccgctgtcaa  | ctctccaact | 60   |
| cagctcagct | gatcggttgc  | cgccgccgcc | gcccgcagat  | tctggaggcg  | aagaacgcaa | 120  |
| agctgagaac | atggacgtta  | atatcgcccc | actccgcgcc  | tgggacgatt  | tcttcccggg | 180  |
| ttccgatcgc | tttgcgccgg  | cggacttcag | ggacatttcc  | aaatggaaca  | accgcgtagt | 240  |
| gagcaacctg | ctctattacc  | agaccaacta | cctgggtgtg  | gctgccatga  | tgatttccat | 300  |
| tgtgggggtt | ctgagtcctt  | tcaacatgat | cctggggagg  | atcgtgggtg  | tgctgggtgt | 360  |
| cacaggggtt | gtgtgggcag  | cccacaataa | agacgtcctt  | cgcgggatga  | agaagcgcta | 420  |
| ccccacgacg | ttcggttatg  | tggtcatgtt | ggcgagctat  | ttccttatct  | ccatgttttg | 480  |
| aggagtcag  | gtctttgtgt  | ttggcattac | ttttcctttg  | ctgttgatgt  | ttatccatgc | 540  |
| atcgttgaga | cttcggaacc  | tcaagaacaa | actgggagat  | aaaatggaag  | gaatagggtt | 600  |
| gaagaggaca | ccgatgggca  | ttgtcctgga | tgccctagaa  | cagcaggaag  | aaggcatcaa | 660  |
| cagactcact | gactatatca  | gcaaagtga  | ggaataaaca  | taacttacct  | gagctagggg | 720  |
| tgagcagaa  | attgagttgc  | agcttgcctt | tgtccagacc  | tatktctctg  | ttgcgttttt | 780  |
| gaaacaggag | gtgcacgtac  | cacccaatta | tctatggcag  | catgcatgta  | taggccgaac | 840  |
| tattatcagc | tctgatgttt  | cagagagaag | acctcagaaa  | ccgaaagaaa  | accaccaccc | 900  |
| tcttattgtg | tctgaagttt  | cacgtgtgtt | tatgaaatct  | aatgggaaat  | ggatcacacg | 960  |
| atctctttta | gggaattaaa  | aaaaataaaa | gaattacggc  | ttttacagca  | acaatacgat | 1020 |
| tatcttatag | gaaaaaaaaa  | atcattgtaa | agtatcaaga  | caatacgagt  | aaatgaaaag | 1080 |
| gctgttaaag | tagatgacat  | catgtgttag | cctgttccct  | atccccctaga | attgtaattg | 1140 |
| gtgggatata | aattagtttt  | tattattctc | ttaaaaatca  | aagatgatct  | ctatcacttt | 1200 |
| gccacctgtt | tgatgtgcag  | tggaactggg | ttaaagccagt | tgttcactat  | tcstttacaa | 1260 |
| atataaagat | agctgttttag | gatattttgt | tacatttttg  | ttaatttttg  | aatgcttagt | 1320 |
| aatgtgtttt | caccagcaag  | tatttggtgc | aaacttaatg  | tcattttcct  | taagatgggt | 1380 |
| acagctatgt | aacctgtatt  | attctggacg | gacttattaa  | aatacaaaaa  | gacaaaaaat | 1440 |
| aaaacaaaaa | aaaaaaaaaa  | gggcggccgc | tctagaggat  | ccckcgaggg  | gccaagcgt  | 1500 |
| ttccngtarg | ttccccttaa  | agacccccg  |             |             |            | 1529 |

&lt;210&gt; 21

&lt;211&gt; 2425

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

<220>  
<221> SITE  
<222> (854)  
<223> n equals a,t,g, or c

<220>  
<221> SITE  
<222> (858)  
<223> n equals a,t,g, or c

<220>  
<221> SITE  
<222> (1155)  
<223> n equals a,t,g, or c

<220>  
<221> SITE  
<222> (1253)  
<223> n equals a,t,g, or c

<220>  
<221> SITE  
<222> (2197)  
<223> n equals a,t,g, or c

<400> 21

|             |             |             |             |             |            |      |
|-------------|-------------|-------------|-------------|-------------|------------|------|
| tcgacccaacg | cgtccgggcta | aatatagacg  | ggagtgccttg | tggtttgatc  | atcaaaggag | 60   |
| ccatgatggg  | tgagaggtgt  | ctagctctga  | atgtcctgtt  | tgctgggggtt | gcttcttgcc | 120  |
| agaggctttt  | cagcaggaat  | cttagctgtc  | actgcttttg  | ggactattgt  | gatccttctc | 180  |
| tctagagctc  | tttcaacgtc  | ctgtgctgac  | tgccagctcc  | ttccagccca  | tcgaaggggg | 240  |
| tccagtgcgc  | ctgaaatgtg  | agacccggct  | ctctccacag  | aggttggatg  | ttcaactcca | 300  |
| gttctgcttc  | ttcagagaaa  | accaggtcct  | ggggtcaggc  | tgagcagct   | ctccggagct | 360  |
| ccagatttct  | gccgtgtgga  | gtgaagacac  | agggtcttac  | tggtgcaagg  | cagaaacggt | 420  |
| gactcacagg  | atcagaaaaac | agagcctcca  | atcccagatt  | cacgtgcaga  | gaatcccat  | 480  |
| ctctaagtga  | agcttgagga  | tccggggccc  | cgggggacag  | gtgactgaag  | gacaaaaact | 540  |
| gatcctgctc  | tgctcagtgg  | ctgggggtac  | aggaaatgtc  | acattctcct  | ggtacagaga | 600  |
| ggccacagga  | accagtatgg  | gaaagaaaaac | ccagcgttcc  | ctgtcagcag  | agctggagat | 660  |
| cccagctgtg  | aaagagagtg  | atgccggcaa  | atattactgt  | agagctgaca  | acggccatgt | 720  |
| gcctatccag  | agcaaggtgg  | tgaatatccc  | tgtgagaatt  | ccagtggctc  | gccctgtcct | 780  |
| caccctcagg  | tcttctgggg  | cccaggctgc  | agtgggggac  | ctgctggagc  | ttcactgtga | 840  |
| gggccttgag  | aggntctncc  | ccaatcttgt  | accaatttta  | tcattgaggat | gtcacccttg | 900  |
| ggaacagctc  | ggccccctct  | ggaggagggg  | cctccttcaa  | cctctctttg  | actgcagaac | 960  |
| attctggaaa  | ctactcctgt  | gaggccaaca  | acggcctggg  | ggcccagtg   | agtgaggcag | 1020 |
| tgccagtctc  | catctcagga  | cctgatggct  | atagaagaga  | cctcatgaca  | gctggagttc | 1080 |
| tctggggact  | gtttggtgtc  | cttggtttca  | ctggtgttgc  | tttgctgttg  | tatgccttgt | 1140 |
| tccacaagat  | atcangagaa  | agttctgcca  | ctaataaacc  | cagaggggct  | tccaggccaa | 1200 |
| atcctcaaga  | gttcacctat  | tcaagcccaa  | ccccagacat  | ggaggagctg  | canccagtgt | 1260 |
| atgtcaatgt  | gggctctgtg  | catgtggatg  | tggtttattc  | tcagggtctg  | agcatgcacg | 1320 |
| cacgccagaa  | agctcagcaa  | acatcaggac  | acttctggag  | aacaaggact  | cccaagtcac | 1380 |
| ctactcttct  | gtgaagaaat  | cataacactt  | ggaggaatca  | gaaggggaaga | tcaacagcaa | 1440 |
| ggatggggca  | tcattaagac  | ttgctataaa  | accttatgaa  | aatgcttgag  | gcttatcacc | 1500 |
| tgccacagcc  | agaacgtgcc  | tcaggaggca  | cctcctgtca  | tttttgtcct  | gatgatgttt | 1560 |
| cttctccaat  | atcttctttt  | acctatcaat  | attcattgaa  | ctgctgctac  | atccagacac | 1620 |
| tgtgcaataa  | aattatttct  | gctaccttct  | cttaagcaat  | cagtgtgtaa  | agatttgagg | 1680 |
| gaagaatgaa  | taagagatac  | aaggtctcac  | ctgcatctac  | tgtgaagtga  | tgagaacagg | 1740 |
| acttgatagt  | ggtgtattaa  | cttatttatg  | tgctgctgga  | tacagtgttc  | taatattttg | 1800 |
| ttgagaattt  | ttgcaaatat  | gttcattggg  | aatattggcc  | tgaaattttc  | ttttccactg | 1860 |
| tgtctctgcc  | agaatgtttg  | tatcaggctg  | atgctggctt  | catagaatga  | gttaggcagg | 1920 |
| agcccttctc  | ccttgatttt  | ttggcatagt  | ttcagcagga  | ttggtaccag  | ttattctttc | 1980 |

|            |            |            |             |            |            |      |
|------------|------------|------------|-------------|------------|------------|------|
| tgcatcttgt | agaattcagc | tatgaatcca | tctgggtctag | ggcttttgtg | ttgggttggt | 2040 |
| agttttttat | tactaattca | acttcagcgc | ttgatattgg  | tctaggaggg | gtttctgtct | 2100 |
| cttcctgggt | caatcttggg | agattgtgtg | tttccaggaa  | tttagccgtt | tcctccagat | 2160 |
| tttcttcttt | atgtgcatcg | acttgagtgt | aaacatnact  | tatatgcact | gggaaaccaa | 2220 |
| aaaatctgtg | tgacttgctt | tattgcagca | tttgttttat  | tttggtagtc | tggaactgaa | 2280 |
| cctgcaatat | caccaaagta | tgcatatagt | tgcaaaaatg  | tgatttttga | catagtaa   | 2340 |
| atgagtattt | gcaataaact | atgatattac | ttttgtaagt  | atatagaata | aaatgtaaat | 2400 |
| aatctaaaaa | aaaaaaaaaa | aaaaa      |             |            |            | 2425 |

&lt;210&gt; 22

&lt;211&gt; 1971

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 22

|            |             |            |             |             |            |      |
|------------|-------------|------------|-------------|-------------|------------|------|
| tgtaagaagg | ctcatgccat  | tgaccctctt | aattctctcc  | tgtttggcgg  | agctgacaat | 60   |
| ggcggagggt | gaaggcaatg  | caagctgcac | agtcagtcta  | gggggtgcca  | atatggcaga | 120  |
| gaccacaaa  | gccatgatcc  | tgcaactcaa | tcccagtgag  | aactgcacct  | ggacaataga | 180  |
| aagaccgaa  | aacaaaagca  | tcagaattat | cttttcctat  | gtccagcttg  | atccagatgg | 240  |
| aagctgtgaa | agtgaaaaca  | ttaaagtctt | tgacgggaacc | tccagcaatg  | ggcctctgct | 300  |
| agggcaagtc | tcagtaaaaa  | acgactatgt | tcctgtatatt | gaatcatcat  | ccagtacatt | 360  |
| gacgtttcaa | atagttactg  | actcagcaag | aattcaaaga  | actgtctttg  | tcttctacta | 420  |
| cttcttctct | cctaacatct  | ctattccaaa | ctgtggcggt  | tacctggata  | ccttggaagg | 480  |
| atccttcacc | agcccccaat  | acccaaagcc | gcacccctgag | ctggcttatt  | gtgtgtggca | 540  |
| catacaagtg | gagaaagatt  | acaagataaa | actaaacttc  | aaagagattt  | tcctagaaat | 600  |
| agacaaacag | tgcaaatattg | attttcttgc | catctatgat  | ggccccctcca | ccaactctgg | 660  |
| cctgattgga | caagtctgtg  | gccgtgtgac | tcccaccttc  | gaatcgatcat | caaactctct | 720  |
| gactgtcgtg | ttgtctacag  | attatgccaa | ttcttaccgg  | ggattttctg  | cttcctacac | 780  |
| ctcaatttat | gcagaaaaca  | tcaacactac | atctttaact  | tgctcttctg  | acaggatgag | 840  |
| agttattata | agcaaatcct  | acctagaggc | ttttaactct  | aatgggaata  | acttgcaact | 900  |
| aaaagaccca | acttggcaga  | ccaaaattat | caaagtgtgt  | ggaattttct  | gtccctctta | 960  |
| atggatgtgg | tacaatcaga  | aaggtagaag | atcagtcact  | tacttacacc  | aatataatca | 1020 |
| ccttttctgc | atcctcaact  | tctgaagtga | tcacccgtca  | gaaacaactc  | cagattattg | 1080 |
| tgaagtgtga | aatgggacat  | aattctacag | tggagataat  | atacataaca  | gaagatgatg | 1140 |
| taatacaaa  | gcaaaatgca  | ctgggcaaat | ataacaccag  | catggctctt  | tttgaatcca | 1200 |
| attcatttga | aaagactata  | cttgaatcac | catattatgt  | ggatttgaac  | caaactcttt | 1260 |
| ttgttcaagt | tagtctgcac  | acctcagatc | caaattttggt | ggtgtttctt  | gatacctgta | 1320 |
| gagcctctcc | cacctctgac  | tttgcacttc | caacctacga  | cctaatacaag | agtggatgta | 1380 |
| gtcgagatga | aacttgtaag  | gtgtatccct | tatttggaca  | ctatgggaga  | ttccagttta | 1440 |
| atgcctttta | attcttgaga  | agtatgagct | ctgtgtatct  | gcagtgtaaa  | gttttgatat | 1500 |
| gtgatagcag | tgaccaccag  | tctcgtctga | atcaagggtg  | tgtctccaga  | agcaaacgag | 1560 |
| acatttcttc | atataaatgg  | aaaacagatt | ccatcatagg  | acctattcgt  | ctgaaaaggg | 1620 |
| atcgaagtgc | aagtggcaat  | tcaggatttc | agcatgaaac  | acatgcggaa  | gaaactccaa | 1680 |
| accagccttt | caacagtgtg  | catctgtttt | ccttcattgg  | tctagctctg  | aatgtggtga | 1740 |
| ctgtagcgc  | aatcacagt   | aggcattttg | taaatcaacg  | ggcagactac  | aaataccaga | 1800 |
| agctgcagaa | ctattaacta  | acaggtccaa | ccctaagtga  | gacatgtttc  | tccaggatgc | 1860 |
| caaaggaaat | gctacctcgt  | ggctacacat | attatgaata  | aatgaggaag  | ggcctgaaag | 1920 |
| tgacacacag | gcctgcaaaa  | aaaaaaaaaa | aaaaaaaaaa  | aaaaaaaaaa  | a          | 1971 |

&lt;210&gt; 23

&lt;211&gt; 1130

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 23

|            |            |            |            |            |            |     |
|------------|------------|------------|------------|------------|------------|-----|
| aattcggcac | gagcttgacc | gccacctgg  | caggagcagg | acaggacggc | cggacgcggg | 60  |
| ccatggccga | gctcccgggg | ccctttctct | gcggggccct | gctaggcttc | ctgtgcctga | 120 |

|            |             |            |             |             |            |      |
|------------|-------------|------------|-------------|-------------|------------|------|
| gtgggctggc | cgtggagggtg | aaggtaccca | cagagccgct  | gagcacgccc  | ctggggaaga | 180  |
| cagccgagct | gacctgcacc  | tacagcacgt | cgggtgggaga | cagcttcgcc  | ctggagtggg | 240  |
| gctttgtgca | gectgggaaa  | cccatctctg | agtcccatcc  | aatcctgtac  | ttaccaaatg | 300  |
| gccatctgta | tccaactggt  | tctaagtcaa | agcgggtcag  | cctgcttcag  | aaccccccca | 360  |
| cagtgggggt | ggccacactg  | aaactgactg | acgtccaccc  | ctcagatact  | ggaacctacc | 420  |
| tctgccaagt | caacaaccca  | ccagatttct | acaccaatgg  | ggtggggcta  | atcaacctta | 480  |
| ctgtgctggt | tccccccagt  | aatcccttat | gcagtcagag  | tggacaaaacc | tctgtgggag | 540  |
| gctctactgc | actgagatgc  | agctcttccg | agggggctcc  | taagccagtg  | tacaactggg | 600  |
| tgcgtcttgg | aactttttcct | acaccttctc | ctggcagcat  | ggttcaagat  | gaggtgtctg | 660  |
| gccagctcat | tctcaccaac  | ctctccctga | cctcctcggy  | cacctaccgc  | tgtgtggcca | 720  |
| ccaaccagat | gggcagtgca  | tctgtgagc  | tgacctctc   | tgtgaccgaa  | ccccccaag  | 780  |
| gccgagtggc | cggagctctg  | attgggggtg | tctgtggcgt  | gctgttgctg  | tcagttgctg | 840  |
| cgttctgcct | ggtcaggttc  | cagaaagaga | gggggaagaa  | gccaaggag   | acatatgggg | 900  |
| gtagtgcact | tccggaggat  | gccatcgctc | ctgggatctc  | tgagcacact  | tgtatgaggg | 960  |
| ctgattctag | caaggggttc  | ctggaaagac | cctcgtctgc  | cagcaccgtg  | acgaccacca | 1020 |
| agtccaagct | ccctatgggc  | gtgtgacttc | tcccgatccc  | tgagggcggt  | gagggggaat | 1080 |
| atcaataatt | aaagtctgtg  | ggtacaaaaa | aaaaaaaaa   | aaaaaaaaa   |            | 1130 |

&lt;210&gt; 24

&lt;211&gt; 1438

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 24

|             |             |            |             |            |             |      |
|-------------|-------------|------------|-------------|------------|-------------|------|
| ggcacgagggc | ggcatgcccc  | ccctccgtca | ccccgcctgg  | ccctgcatct | tctcactgct  | 60   |
| catgggcatc  | agcaacgggt  | acttcggcag | cgtgcccattg | atcctggcgg | caggcaaaagt | 120  |
| gagccccaag  | cagcggggagc | tggcagggaa | caccatgacc  | gtgtcctaca | tgtcagggct  | 180  |
| gacgttgggg  | tccgcctgtg  | cctactgcac | ctacagcctc  | acccgcgacg | ctcagcgagc  | 240  |
| ctgcctgcac  | gcctccaccg  | ccaatgggtc | catectcgca  | ggcctctgag | ccagccccgc  | 300  |
| ccactgccag  | ggacgcccag  | ggcctgacca | ggggccccga  | ggcctgaggg | ccccccccct  | 360  |
| gtccccacct  | cagtgcctgc  | ggggccctga | gcctccccct  | gtgccagcag | ccccactccc  | 420  |
| tcaggggtcca | gcatgcccc   | accctggact | gaagtctctg  | aaagtccctc | gaggaccgga  | 480  |
| acacgtttct  | gcgaccggg   | gctctggcca | gcactgtgtt  | ctgcgttttg | tctcatacct  | 540  |
| gcgtctacct  | tccatctgtg  | tccagcggcc | ccggctccag  | cccagccagc | actctgcagg  | 600  |
| gtcacacgca  | ccgtgtcccc  | acccaggaca | gcagacaccc  | gccagagtgt | gcgcgcccag  | 660  |
| tgactgcacc  | ccggccctca  | tcacccaccg | gcactgattg  | gggcaccgcc | tggcccagcc  | 720  |
| tccaccaggg  | acccctcctc  | atgaactctg | gagccctgag  | aggagagggg | cagcccccca  | 780  |
| ccttgtcacc  | ctcagggctt  | ccccctctgt | cctcattctt  | agagactgct | tctcccaaac  | 840  |
| ataacgcgtt  | agccatgaag  | gagtcggagc | cctgggtccg  | aatggaccgg | cctgcggtct  | 900  |
| gcacagcct   | ctgggaaacc  | acagcagtga | tgccagctgg  | gcacgtcagg | acctccccac  | 960  |
| acaccacac   | gatgccacag  | gtcagggggc | tgtgcctgac  | tagggagccc | tccattggcc  | 1020 |
| ttcctggccc  | gggatagaag  | aggggaggta | agtctggggg  | ctacgaagcc | gggccccac   | 1080 |
| accctggctg  | aagtcagctt  | gacctaggtc | ttgacctca   | tccagcaagg | gactcgacag  | 1140 |
| acccaagggt  | ccctggaacg  | tagggagggg | ctgggggtca  | ctccagcccc | ggcctcccag  | 1200 |
| aacaccaggc  | ccgtgtgggt  | ggcaccctga | ggtcagggga  | tcctaagggt | gtccttcag   | 1260 |
| agacggtgtt  | tccaggggga  | ggaccgcccc | cgcttcagga  | tccccggccc | cggtgtgac   | 1320 |
| tgccctgttt  | caccctgtgt  | gtgtcccatc | ccccgtctgt  | ccactaactg | taccgcaccg  | 1380 |
| gccattaaaa  | gatgaaggca  | gaccgctgca | aaaaaaaaa   | aaaaaaaaa  | aaaaaaaaa   | 1438 |

&lt;210&gt; 25

&lt;211&gt; 916

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (499)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 25

|             |            |            |            |            |             |     |
|-------------|------------|------------|------------|------------|-------------|-----|
| gagcaaaacc  | acactgaaat | gcaccccact | ccaggagagg | aattcttagc | gtaacactct  | 60  |
| aaataaatgg  | aaggaatcat | caccttcctt | atcttacccc | tgccttggtc | accaggctgc  | 120 |
| ccagtgccta  | ccatgcagaa | agcagtcagc | tgtactctgg | aagtttctgt | tcttctttcc  | 180 |
| tggggccttag | gatattctgg | gagctgtctg | agccttggtc | ctaaggctta | tcagggtgata | 240 |
| taatcttcct  | gttctgggct | gcttgctgga | ggaataggaa | gtgacattta | taagacacag  | 300 |
| gcggtgtgag  | catccatgtg | tggctctggg | ctaaaccagc | tcttgaacag | gttaaagcaa  | 360 |
| acagcaataa  | caaaacaaaa | actactgatg | ctgagcggtt | tgatcctagt | aatatttcaa  | 420 |
| atattgtcct  | tctgcatatg | tyctatccat | attgattcca | atatmcatta | taagcttctt  | 480 |
| gggtactawt  | tgtctgggnc | tcttscgtga | argtggtacc | tgtctcatga | tccttaaaag  | 540 |
| agagaggytt  | ttttcatcca | aagctgtagt | gttggaact  | ggggtgggag | aggcactttt  | 600 |
| tgggaattctg | aaagaatcat | atctgtgtat | atacatactg | agtggggaag | gatgggggtt  | 660 |
| ggcagggggtt | gagggaggtg | ggaacaaaca | gtgagtatgg | gaacaggcag | tcacctcgag  | 720 |
| tgtgggaggt  | cacctgggtc | cgctgtcttc | cttctgtatg | gtgttgggtt | tatgtacaca  | 780 |
| ctataacact  | tcctgtgtga | gttcatgtac | ctgtctgtga | gtgctttggt | gtattgagcc  | 840 |
| tcagtacact  | ccaagggcat | taaagtcaag | aactagaaaa | aaaaaaaaaa | aaaaaaaaaa  | 900 |
| aaaaaagggc  | ggccgc     |            |            |            |             | 916 |

&lt;210&gt; 26

&lt;211&gt; 2094

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (2078)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 26

|             |            |            |             |             |             |      |
|-------------|------------|------------|-------------|-------------|-------------|------|
| gaggggttga  | agccgcgccg | cgagggagcg | aggctgcagt  | gacagcggcg  | ggcgatcgga  | 60   |
| cccaggctgc  | cccgcgtac  | ccgcctgcgt | cccgcgtcc   | cgccccagca  | tgacagcccc  | 120  |
| ggcggktccg  | cgcggtcag  | agaccgagcg | gcttctgacc  | cccaaccccg  | ggtatgggac  | 180  |
| ccaggcgggg  | ccttcaccgg | ccctccgcac | acccccagaa  | gaggaagacc  | ttcgccgtcg  | 240  |
| tctcaaatac  | ttttcatga  | gtccctgcga | caagtttcga  | gccaagggcc  | gcaagcctgc  | 300  |
| aagctgatgc  | tgcaatggtc | aagatcctgg | tggctacggg  | gcagctcatc  | ctgtttgggc  | 360  |
| tcagtaatca  | gctggctgtg | acattccggg | aagagaacac  | catcgccctc  | cgacacctct  | 420  |
| tctgtctggg  | ctactcggac | ggagcggatg | acaccttcgc  | agcctacacg  | cgggagcagc  | 480  |
| tgtaccaggc  | catcttccat | gctgtggacc | agtacctggc  | gttgccctgac | gtgtcactgg  | 540  |
| gccggtatgc  | gtatgtccgt | ggtgggggtg | acccttggac  | caatggctca  | gggcttgctc  | 600  |
| tctgccagcg  | gtactaccac | cgaggccacg | tggaccggcg  | caacgacaca  | tttgacattg  | 660  |
| atccgatggt  | ggttactgac | tgcattccag | tggatcccc   | cgagcggccc  | cctccgscce  | 720  |
| ccagcgcagc  | tctcaccctc | ttggaaagca | gctccagtta  | caagaacctc  | acgtcaaat   | 780  |
| tccacaagct  | ggtcaatgtc | accatccact | tccggctgaa  | gaccattaac  | ctccagagcc  | 840  |
| tcatacaata  | tgagatcccc | gactgctata | ccttcagcgt  | cctgatcacg  | tttgacaaca  | 900  |
| aagcacacag  | tgggcggatc | cccatacagc | tggagaccca  | ggcccacatc  | caggagtgtg  | 960  |
| agcaccaccag | tgtcttccag | cacggagaca | acagcttccg  | gctcctgttt  | gacgtgggtg  | 1020 |
| tcatacctac  | ctgctccctg | tccttccctc | tctgcgccc   | ctcactcctt  | cgaggcttcc  | 1080 |
| tgctgcagaa  | ygagtttgtg | gggttcatgt | ggcggcagcg  | gggacgggtc  | atcagcctgt  | 1140 |
| gggagcggct  | ggaatttgtc | aatggctggt | acatcctgct  | cgccaccagc  | gatgtgctca  | 1200 |
| ccatctcggg  | caccatcatg | aagatcggca | tccaggccaa  | gaacttggcg  | agctacgacg  | 1260 |
| tctgcagcat  | cctcctgggc | acctcgacgc | tgtctggtgtg | ggtgggcgtg  | atccgctacc  | 1320 |
| tgaccttctt  | ccacaactac | aatatcctca | tgcacacact  | gcgggtggcc  | ctgcccagcg  | 1380 |
| tcatgcgctt  | ctgctgctgc | gtggctgtca | tctacctggg  | ctactgcttc  | tgtggctgga  | 1440 |
| tcgtgctggg  | gccctatcat | gtgaagtcc  | gtcactctc   | catggtgtct  | gagtgccctgt | 1500 |
| tctcgtcat   | caatggggac | gacatgtttg | tgacgttcgc  | cgccatgcag  | gcgcagcagg  | 1560 |
| gccgcagcag  | cctgggtgtg | ctcttctccc | agctctacct  | ttactccttc  | atcagcctct  | 1620 |

|             |             |             |             |            |            |      |
|-------------|-------------|-------------|-------------|------------|------------|------|
| tcattctacat | gggtgctcagc | ctctttcatcg | cgtctcatcac | cggtgcctac | gacaccatca | 1680 |
| agcatccccg  | cggtgcaggg  | gcagaggaga  | gagagctgca  | ggcctacatc | gcacagtgcc | 1740 |
| aggacagccc  | cacctccggc  | aagttccggc  | gcgggagcgc  | tcgggcctgc | agccttctct | 1800 |
| gctgctgcgg  | aagggacccc  | tcggaggagc  | attcgctgct  | ggtgaattga | ttcgacctga | 1860 |
| ctgccgttgg  | accgtagggc  | ctggactgca  | gagacccccg  | cccccgacce | cgcttattta | 1920 |
| ttttagagggt | ttgcttttaa  | ggatcggctc  | cctgtcgcgc  | ccgaggaggg | cctggacctt | 1980 |
| tcgtgtcgga  | cccttggggg  | cggggagact  | gggtggggag  | ggtgttgaat | aaaagggaaa | 2040 |
| ataaaaaaaaa | aaaaaaaaaa  | aaaaaaaaaa  | aaaaaacnty  | kggggggggc | ccct       | 2094 |

&lt;210&gt; 27

&lt;211&gt; 2076

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 27

|            |            |             |             |             |            |      |
|------------|------------|-------------|-------------|-------------|------------|------|
| ccacgcgtcc | gctccagtcg | gcgtctcagc  | agtctccggg  | gtccacgcgt  | tgactgccgt | 60   |
| gtgtgactcc | agtcggcgtc | tcagcagtc   | cggtgtccca  | cggttgact   | gocgtgtgtg | 120  |
| actccagtcg | gcgtctcagc | agtctccggg  | gtccacgcgt  | tgactgccgt  | gtgtgactcc | 180  |
| agtcggcgtc | ttaacagtct | ccggtgtcca  | tgcttgact   | gccatgtatg  | actccaggta | 240  |
| ttgagttcat | cttcaacttc | tcccaacaat  | gaactggagt  | tttctgtgca  | tgtgtctggc | 300  |
| atgttttcc  | ttagatctag | tcctaggtgt  | tcgatatgct  | attgaggact  | gtgtattttt | 360  |
| atttcatttg | tcacctgtgc | gtgggtcctt  | aatcctgtgc  | cctaaactcc  | cgcttggcc  | 420  |
| atggcgggtg | ttctgtggac | tcgtgggggt  | tcctgtgca   | cacgcgtgtc  | ctctgtctga | 480  |
| cagtggcttt | gcttccccct | gtcagtcctg  | gcctcggctt  | ctcactgcac  | tgccgaggtg | 540  |
| agccggcgct | tgctaactct | attccagtc   | cggtgaacat  | gggtcagtc   | tttcccggt  | 600  |
| cagtgttggg | tttgactgg  | tgcaacttaca | ggggaagagc  | ttcctcattt  | gctgagggtc | 660  |
| tttctgaat  | ccgtgttgaa | tggttcagtg  | cctttcctgc  | acctagttag  | atgtcatga  | 720  |
| ggtcacttcc | ttaccgcatt | actgtagtga  | attacgctga  | ctttcatatg  | ccaagatgac | 780  |
| ctggcatttc | aggtaagtc  | ctgtggccat  | gggtgcattg  | ccttttctct  | gcatggccag | 840  |
| atgagatttg | ctcataccgg | gctaaggacc  | tcctgtgtag  | gaatgctctt  | gagggacact | 900  |
| gggcttggat | gttcattgtc | ttgtcatgtg  | atgtgtgtgt  | catggttgat  | attgcattaa | 960  |
| gtgggcctcc | tgaaacaagt | tcctgcatag  | tttgtatagg  | attggcatta  | tttgtttcat | 1020 |
| agagatgtga | tggttgaggc | catctatgcc  | tgccattttc  | tttttgctta  | ggtttttcat | 1080 |
| taagaacttg | atttttataa | atagacatga  | ggcctggcat  | ggtggctcac  | gcttgtaate | 1140 |
| ccagcacttt | gggaggccaa | cgtggatgga  | tcacctgagg  | tcaggagttt  | gagaccaacc | 1200 |
| tagtcaacat | ggtgaaaccc | cgtctccatt  | aaaaatataa  | aaattagcag  | ggtgtgggtg | 1260 |
| tgcatgcctg | taatcccagc | tactctggag  | gctgaggcag  | gagaatcact  | tgaactcagg | 1320 |
| agggtggagg | tgcaagtggc | tgagatcaca  | ccactgcact  | ccagcctggg  | caacagagcg | 1380 |
| aggctctgtc | tcaaaaaaaa | agacatgagg  | cttttggatt  | ttctgtttct  | tcctttgtca | 1440 |
| ttttttttaa | gataggtttg | tcgcagaatt  | tcattctaagt | tatcaaaaaca | atttacataa | 1500 |
| atcattcaca | gtatcctctg | tgcaagtgat  | tccattctca  | ttcctgctct  | gggccatttg | 1560 |
| tgttttcttt | tccccctgat | cagactagct  | aagagtttat  | cattttttaca | gatattttcc | 1620 |
| ccaaagaaac | ctacttttgg | gtccattgat  | tttttttttc  | tattgttttc  | tttttcattg | 1680 |
| acttttgctc | ttttcttatg | aatttccctt  | cttctaccta  | ttttgtgttc  | aatttgctgt | 1740 |
| ccttgtttta | ggttcttttg | atagaggctt  | agatcattga  | tgaagaatat  | gaaagaatgg | 1800 |
| gataatacag | ttaaaaactt | ggtctactta  | tgccatttag  | agaaaacatt  | cttaagcacg | 1860 |
| catggaatct | tttaaaaatg | gacgaggtac  | taggacccaa  | agcaaatgtc  | agtaattcgc | 1920 |
| agatgataat | gtaatgtata | aatatatgat  | atgtaggtaa  | ataatacaaa  | tcacattctt | 1980 |
| tgccagaggt | gcaactgagg | aggaaatcag  | taacaaaaag  | acaattaaat  | ccatttgatt | 2040 |
| cttttgaaaa | aaaaaaaaaa | aaaaaaaaaa  | aaaaaaa     |             |            | 2076 |

&lt;210&gt; 28

&lt;211&gt; 1378

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 28

|            |             |             |             |            |            |      |
|------------|-------------|-------------|-------------|------------|------------|------|
| ggcacgaggt | cctttgtgac  | tgatgtcttt  | cacttagcat  | ggtgtttaca | aggttcatcc | 60   |
| atgtttagat | atgtatctac  | ttaatttttt  | tctatgattg  | aataatagtc | cattgtatga | 120  |
| aaatacaaca | ttttatttat  | ctactcatca  | ggtgatggcc  | atttgggttg | ttttcatcta | 180  |
| ttggctatta | ttagtcttct  | gtgaacactc  | atgtataagt  | tttcgtgtgg | atgtatgtat | 240  |
| tcatttctct | tgtatacaaat | tctacctggg  | agtgggaattg | ctggatcata | tggcggctct | 300  |
| tttgacctta | tggggaactg  | ctagattggt  | attcaagggtg | tctgcacctt | gttcattatc | 360  |
| atcagcagtt | tatgatgggt  | ctgtttcatc  | acaacctcac  | caatacttat | tttctgtctg | 420  |
| taggtggggg | ttgttggaac  | accaccacat  | tcattcattt  | acgtattatc | tatggctgct | 480  |
| tttgcagtac | agctagagag  | ttgaatagtt  | atggatacag  | actgtatgac | tcaaaaggcc | 540  |
| caaaatattt | actaattggc  | ccttcacaga  | aaaagtttgc  | tgctagaggt | atgttataaa | 600  |
| gtgaagttag | tcattgttata | tttgaatgcc  | tgggtgaagt  | atgctaatac | aggaataggc | 660  |
| tagatatctt | tcgagacttc  | ctaagtggcc  | tcacagaacc  | tatagtcact | taaaccaaag | 720  |
| tatacttgta | tggccagtat  | cttagctatg  | caattatata  | tgtagacag  | aaattctatg | 780  |
| attaaaaaag | agaggcaatt  | cattgtcata  | atttgtcatc  | aaaagcatta | taacaaatta | 840  |
| taaaaatact | agaaaaggta  | ggtatagtat  | caaaatgata  | aataccagta | aacatgatgc | 900  |
| tccagaacat | gtaaaaatata | tattttggag  | tatcatagca  | tagcacgggg | aggggaaaat | 960  |
| taagtttgca | agtcaagaat  | agtgtggggg  | agggagtttg  | gagggaaaag | acaagttggg | 1020 |
| tagacttttg | aggatacggc  | aggtgctaaa  | tcttagaaga  | atctgcaatg | ctcacttggt | 1080 |
| tcccagaagt | agctacaccc  | aagtcaaccc  | ctggcttggt  | ttccactaac | ggagccaaat | 1140 |
| acaaactggg | gtggctacag  | atatggaaaag | ctggtatcaa  | gatcttattt | ttgataagta | 1200 |
| aaaaggacta | tacatgagga  | gtgccatcaa  | gatggggatg  | gggtgactgg | gaggaggaag | 1260 |
| agaaaggtga | gtcagtcattg | cacaaaaactt | aattattttg  | gctcctcagc | agttttgttt | 1320 |
| gtcaccaagt | gtgtgtagac  | aaataacatt  | aaatgctctt  | aaaaaaaaaa | aaaaaaaaa  | 1378 |

&lt;210&gt; 29

&lt;211&gt; 1965

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 29

|            |            |            |             |             |             |      |
|------------|------------|------------|-------------|-------------|-------------|------|
| aagccaagct | tggcacgagc | aggacatggc | tcagcttggtg | cccagagggga | gagttaagcc  | 60   |
| gctgacctg  | tagccagggg | gtgcacctgc | aagcatgggg  | gtggcaggag  | ccacagagct  | 120  |
| ggctgctgag | aggagctgca | gatctggaga | agacagccta  | ggtaaagggtg | gacagtgtga  | 180  |
| gagctgctga | ttagatagct | gctgaataaa | actacatttt  | acctgcctat  | ggcccgccag  | 240  |
| gttttctttc | agctatcgcc | catccacca  | gtccctcga   | acctcagcat  | gggctggaac  | 300  |
| ctgacctgg  | gcatgacatt | tggcatagtt | gtggacctga  | cacctgtgtt  | tgtcctagtc  | 360  |
| ctgtttctcc | ctgccttctc | gttctctctg | ctgcctctat  | ggtcactccc  | aagagatcca  | 420  |
| acccatgtta | agtatgggct | ggaggactgc | atgaatgcct  | catgatcttc  | ccagaggcaa  | 480  |
| aggcacctac | tgccttccaa | ggtcagtggg | aggttgggat  | caacactggt  | tattatgctt  | 540  |
| aggacaaaaa | agatggggag | aaagatgtgc | aaccttacag  | gtcatctttc  | tgggatagaa  | 600  |
| cacaatgggt | cttctctctg | ctcctggata | tgtagtcaa   | ggccagtcca  | tgctacacat  | 660  |
| ctagtctgac | ttctaaaata | gaagcaccag | atgaattcag  | ccctgagaga  | attttcagca  | 720  |
| gctgtggggg | cgctggagga | aacactatta | aatagttttg  | cacctgagac  | agatagcctc  | 780  |
| actcgctca  | ccctagtcct | ggtggcattt | gtctcagggtg | caaaatttaa  | gaaagaaacc  | 840  |
| ttggagtgtc | cacctgtggg | ctgggtagat | ggtcctaaag  | tgggtggtttt | caagcctgag  | 900  |
| tgtgtatcag | gatcatcagg | ggagcttgct | aaagagcagt  | tctgcggtc   | agaccctcat  | 960  |
| gcattttgag | caggtgtggg | gactgggaaa | ctgcatctgt  | aacctgctgt  | aatctaacgc  | 1020 |
| ttatctaaat | actactgtgc | tcacacagag | aacaccgcaa  | aagtagaggt  | gttctctccag | 1080 |
| agggcagggt | agcagatggc | acagtctgct | tggaaattcag | tcaggtgatg  | agagatgaga  | 1140 |
| tgaggcactc | ctagcttttg | gaagagggag | ctgaaagatg  | aacctttgca  | ggtgccacg   | 1200 |
| gtcaaagtgg | tggtttaagt | ccatgccatg | cccattttct  | gttggccttg  | gcaggaggtt  | 1260 |
| acagccctac | cttaggacct | ggctccttat | ttctgctgta  | ggctctttcc  | tgccctggcc  | 1320 |
| gagatggagt | ggaatgagcc | tagaacatca | gctaatacat  | gtcctcagaa  | agataaaggt  | 1380 |
| ttacattttc | acccccatca | aatctgaaag | ctctctgcct  | gtgtttttct  | aagggatagg  | 1440 |
| gacatcatta | ctcagtcac  | aacctggact | catgtagggt  | cccctgtcag  | taaaggagtc  | 1500 |
| agtcaagccc | accaggtata | ccaaggactc | ttacctcag   | cccctactcc  | ttggaaagct  | 1560 |
| gcccttggtc | ctaattattg | tgtttagctt | gagcctgact  | ccttctcaac  | actaagagct  | 1620 |
| gatgaagtcc | tgaagcagaa | agagctctga | cctgagagtc  | aaacatcctt  | attctgatct  | 1680 |

cagctcagcc cctgatttgc tgtgtgaccc tggatatgtc acttcctgtc tttttgactt 1740  
tttaaaatga agggtagact agaggagagc ttctaaaact ttaatgtggc caacgaaatg 1800  
gaataggaaa ttccacaagt ctgtccttcc acaaaagcag caaataaggc ggcaaaaact 1860  
caaatttatg ggaactctgg aaacgaattg aaagtttaca gcaatcaggc gaatacctaa 1920  
gaataaaaagc tggattttagt aagaaaaaaa aaaaaaaa aaaaa 1965

&lt;210&gt; 30

&lt;211&gt; 1473

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 30

gaattcggca cgagccgcgg ctgagggcag ggggtcgcctg cgaaccccg ggttccgcgg 60  
tggaggggtg ctatactggg atgcaggcgc ggccggggact ggcagcaatc atgccctggg 120  
agctaacgta gagcttttga taatgctttt gcaagttgta cgagaaggga agttctcggg 180  
gtttctgacc tcctgcagcc tcctcttggc tcgggctgccc cagatcttgg cggctgaggc 240  
tggcttacct tcgagccgtt ccttcattggg atttgcctgt cccttcacca acaagcgaaa 300  
ggcttactcg gagcgtagaa tcatggggta ctcaatgcag gagatgtatg aggtgggtgtc 360  
caacgtccag gaggatcgtg agttttgtgc ctgggtgaag aagtctctgg tggatatccag 420  
ccgtaagggc catttgaaag ccagctgga ggttggcctt ccacctgtca tggaaacgtta 480  
cacctctgca gtttccatgg tcaaacctca catggtcaag gctgtttgta ctgatggcaa 540  
gctcttcaac cacttagaga ctatttggcg attcagccct ggtattcctg cctatcctcg 600  
aacctgcact gtggactttt cgatttcctt tgaatttcgt tctctgctgc actcccagct 660  
ggccaccatg ttttttgatg aggttgtcaa acagaatgtt gctgcctttg agcgtcgggc 720  
agccaccaag tttgggtccag aaacagccat ccccggtgaa ctgatgttcc atgaggtgca 780  
ccagacttga ggcaagggat tgctccctga cctcccttct accccacttc cctacacaat 840  
tctcttattt atttggtttg gctcctgttc caatttgaaa ggagtctgtg ttcataatac 900  
tgtttcycct ctcaatttcc cagaaatttg gttctatgct ggctggaaat gttgggggaa 960  
agagaaggca aaggatgtgg aaatgagatg tgcttaggaa agggctcaggc ccacgtagg 1020  
agcaccatat gcctgcagcc ttttccactac gaattagaat aaggactatg tggttgtctc 1080  
tggaccttat caagacacct tagtgtctga ccaggggacg atagtaactt ttctaaggat 1140  
tgaataaatt gagcttttct tctggcacag aggtactgag tggtaagtaa cttttaccct 1200  
gcctgagatt cctcaggaga aaaggcaacc tgcctccagc ctgaaatata taaagcctca 1260  
ttttaagact gtaagtccat gctgcctggc tactagagag caaggggctt tcttaccacc 1320  
agtgtgtagg agaaaagtac tgaacggaaa cggagttgtc tttgtactct tgagttgtac 1380  
cttattcttc cacttggcct gagtttttat aaaatttcaa taaattgtga cagtgtgaaa 1440  
aaaaaaaaa aaaaagatct ttaattaagc ggt 1473

&lt;210&gt; 31

&lt;211&gt; 1157

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 31

gagatggatt ttcttccaaa aatgcagacc cattttaatt aagtttgtaa ttaaccactg 60  
gggagggcag gccccctgga ttcgggtctgc tttcggagac actatgggag tcatggccat 120  
gctgatgctc cccctgctgc tgctgggaat cagcggcctc ctcttcattt accaagaggt 180  
gtccaggctg tggcctaaagt cagctgtgca gaacaaagtg gtgggtgatca ccgatgccat 240  
ctcaggactg ggcaaggagt gtgctcgggt gttccacaca ggtggggcaa ggctgggtgt 300  
gtgtggaaag aactgggaga ggctagagaa cctatatgat gccttgatca gcgtggctga 360  
ccccagcaag acattcaccc caaagctggt cctgttggac ctctcagaca tcagctgtgt 420  
cccagatgtg gcaaaagaag tcctggattg ctatggctgt gtggacatcc tcatcaacaa 480  
tgccagtgtg aagggtgaag ggccctgccc taagatttct ctggagctcg acaaaaagat 540  
catggatgcc aattactttg gcccacatcac attgacgaaa gcctgtcttc ccaacatgat 600  
ctcccggaga acaggccaaa tcgtgttagt gaataatata caaggggaagt ttggaatccc 660  
gttccgtacg acttacgctg cctccaagca cgcagccctg ggcttctttg actgcctcgg 720  
agccgaagtg gaggaatacg atgttgtcat cagcaccgtg agcccgactt tcatccggtc 780



|            |            |            |             |            |             |      |
|------------|------------|------------|-------------|------------|-------------|------|
| gtaccacgtg | tatccagagc | aaggaaactg | ggaagcttcc  | atttggaat  | tctttttcag  | 840  |
| gaagctgacc | tacggcgtgc | acccagtara | ggtggcggag  | gaggtgatgc | gcaccgtgcg  | 900  |
| gaggaagaag | caagaggtgt | ttatggccaa | ccccatcccc  | aaggccgccg | tgtacgtccg  | 960  |
| caccttcttc | ccggagtctt | ttttcgccgt | ggtggcctgt  | ggggtgaagg | agaagctcaa  | 1020 |
| tgtcccgagg | gaggggtaac | tgcaggaggc | caaatgggccc | accccttgga | aataaagggtt | 1080 |
| tttctggcaa | aaaaaaaaaa | aaaaaaaaaa | aaaaaaaaaa  | aaaaaaaaaa | aaaaaaaaaa  | 1140 |
| aaaaaaaaaa | aaaaaaa    |            |             |            |             | 1157 |

&lt;210&gt; 32

&lt;211&gt; 2190

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 32

|             |             |             |             |             |             |      |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| tgcacccacg  | cgtccggggc  | tgccctctcca | ctcagagggga | catcagctca  | agagcaatca  | 60   |
| cacaacccca  | gagaagaaat  | ccaaacctca  | ccttctgttg  | ctgcttctct  | actatcttat  | 120  |
| gggtgcttga  | ctggctctcc  | caagcctgct  | gtcctgcagc  | ctcactgcct  | gtgtcctttt  | 180  |
| ctcaggcagt  | gtgctggcgt  | tccatgcgac  | gtggctgtgc  | agtgtctgga  | gccctggggc  | 240  |
| tgctggccgg  | tgcaggtgtt  | ggctcatggc  | tcctagtgtc  | gtatctgtgt  | cctgtctgct  | 300  |
| ctcagcccat  | ttccgggacc  | ttgcaggatg  | aggagataac  | tttgagctgc  | tcagaggcca  | 360  |
| gcgctgagga  | agctctgtct  | cctgcacttc  | ccaaaacagt  | atcttttcaga | ataaacagcg  | 420  |
| aagacttctt  | gctggaagcg  | caagtgaggg  | atcagccacg  | ctggctcctg  | gtctgccatg  | 480  |
| agggctggag  | ccccgccttg  | gggctgcaga  | tctgtggag   | ccttgggcat  | ctcagactca  | 540  |
| ctcaccacaa  | gggagtaaac  | ctcactgaca  | tcaaactcaa  | cagttcccag  | gagtttgctc  | 600  |
| agctctctcc  | tagactggga  | ggcttctctg  | aggaggcgtg  | gcagcccagg  | aacaactgca  | 660  |
| cttctgggtca | agttgtttcc  | ctcagatgct  | ctgagtggtg  | agcgaggccc  | ctggcttccc  | 720  |
| ggatagtttg  | tgggcagttc  | gtggctcctg  | ggcgctggcc  | gtggcaggcc  | agcgtggccc  | 780  |
| tgggcttccg  | gcacacgtgt  | gggggctctg  | tgctagcgcc  | acgctgggtg  | gtgactgctg  | 840  |
| cacattgtat  | gcacagtttc  | aggctggccc  | gcctgtccag  | ctggcggggt  | catgcggggc  | 900  |
| tggtcagcca  | cagtgcctgc  | aggccccacc  | aaggggctct  | ggtggagagg  | attatccac   | 960  |
| acccctcta   | cagtgcctgc  | aatcatgact  | acgacgtcgc  | cctcctgagg  | ctccagaccg  | 1020 |
| ctctcaactt  | ctcagacact  | gtgggcgctg  | tgtgcctgcc  | ggccaaggaa  | cagcattttc  | 1080 |
| cgaagggctc  | gcgggtgctg  | gtgtctggct  | ggggccacac  | ccaccctagc  | catacttaca  | 1140 |
| gctcggatat  | gctccaggac  | acggtgggtg  | ccttgttcag  | cactcagctc  | tgcaacagct  | 1200 |
| cttgcgtgta  | cagcggagcc  | ctcaccctcc  | gcattgcttg  | cgctggctac  | ctggacggaa  | 1260 |
| gggctgatgc  | atgccaggga  | gatagcgggg  | gccccctagt  | gtgcccagat  | ggggacacat  | 1320 |
| ggcgccctagt | gggggtgggtc | agctgggggc  | gtggctgcgc  | agagcccaat  | caccaggtg   | 1380 |
| tctacgcca   | ggtagctgag  | tttctggact  | ggatccatga  | cactgctcag  | gactccctcc  | 1440 |
| tctgagtcct  | gctgtttcct  | ccagtctcac  | tgcacaccac  | tgctcctatg  | ttcctggggc  | 1500 |
| ctccagcagc  | tccactaatg  | gaggagaggc  | agtagcttcc  | gacacagaac  | gcattggacct | 1560 |
| cctactactg  | tgtgtgagga  | acagtcacta  | cccactggcc  | agccaccag   | ccaacaggtc  | 1620 |
| tctcctcttg  | ggccctgatt  | tcagagtcc   | ctttctcact  | agagactcaa  | tgacagaaga  | 1680 |
| gaggctggga  | cttgggttggg | catgctgtgg  | ttgatgaggg  | atgaggggga  | ggagagaggt  | 1740 |
| aggagctgga  | gatgaagagg  | ctgctagaag  | cagcaggaag  | cctgcccttc  | tgccctctcc  | 1800 |
| cttccctgcc  | cctgtgtgag  | tcttttggga  | gggtgctggg  | aggtgcccc   | cgtcccacct  | 1860 |
| tttctctgtg  | ctctaggttg  | gctaagtgcc  | tccctagagg  | actccatggc  | tgagaggctc  | 1920 |
| ctgggcagat  | gggggtcaagg | ctgggcccagc | ccagatgaag  | cctatggggag | tcaggaccct  | 1980 |
| ctccactctc  | cctctccact  | ccccttccctg | ttctcacctg  | gctgtggctg  | gccctgtgtg  | 2040 |
| gggtgggttac | actggaaaac  | aagaaggttg  | gagttgggtc  | aggacattgg  | ttttaaatga  | 2100 |
| cagttctgtg  | aactgggtcca | aggagtctctg | ttattaaagt  | gatatatggt  | cttgggtccaa | 2160 |
| aaaaaaaaaa  | aaaaaaaaaa  | aaaaaaaaaa  |             |             |             | 2190 |

&lt;210&gt; 33

&lt;211&gt; 2511

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 33

|             |             |             |             |             |             |      |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| ctgcaggaat  | tccggcacgag | catttttcagg | cctcagtatg  | gtggccttatt | ctgtccaggt  | 60   |
| tctagccgta  | tttatcagct  | gtgcaatatt  | aacccttgca  | atgaaaatag  | cttggatttt  | 120  |
| cgggctcaac  | agtgtgcaga  | atataacagc  | aaacctttcc  | gtggatgggt  | ctaccagtgg  | 180  |
| aaacctata   | caaaagtgga  | aggtaatttg  | gtctctatga  | cattcaaata  | agtctctatg  | 240  |
| tggataataa  | tgtgattgac  | caatacagaa  | gaaattgctg  | cttgtatcaa  | agagtttgaa  | 300  |
| tgtgtacctt  | tcactcccca  | gacctatcct  | ggctcagtg   | ttcattccat  | gggttttggt  | 360  |
| tatttttcca  | ctgttggttt  | tttttttccc  | cctataagat  | acctcttgct  | tcccacatct  | 420  |
| gtaaacccaa  | ggtttcatat  | gttttgaatg  | aactcatgtt  | tctcaactta  | cctttgacat  | 480  |
| ttgggcaatt  | aagtctccca  | gggatcttaa  | ttgaaattgg  | tatgaactgt  | ccagaccaaa  | 540  |
| aactcatgaa  | accatatata  | ccccatagag  | gcaccgtaca  | cagtaactga  | catgtcagtt  | 600  |
| gattacaagc  | atgggaaggg  | ctgtgtgtag  | agtgtgtgca  | tgcacgcaga  | cacacacaca  | 660  |
| gaatcataaa  | taaaaattat  | actaggttct  | tggtacttac  | tatacttaa   | gttatggctt  | 720  |
| atattttact  | ttttaaatata | atgtgtcttt  | attttgactg  | aataataaaa  | cattagttac  | 780  |
| agcagaagag  | tttaatgtag  | ttgtctttac  | ctatgtagaa  | taagctgtca  | tcatcctagt  | 840  |
| tgtttcatta  | cttccatcca  | ttagatttat  | caaaactggt  | acgtgtgctt  | gaaacagggg  | 900  |
| tatatgtgaa  | tcccaactgc  | ccatgtgtag  | aatggaaaga  | gactaagttt  | aatgacaatc  | 960  |
| catgggctgt  | tctgtgcaaa  | agcgcaacat  | gaacctatat  | taatcagtga  | tcataaagga  | 1020 |
| agctaataata | attacaggca  | tcaactatac  | aagtagagcc  | tccaaatcga  | aagagaggat  | 1080 |
| tgtgcagctt  | tgctcagtat  | cagcctagcc  | atttcaggag  | caactgatgtg | gttttgggtc  | 1140 |
| atgactgttt  | gccaggaagt  | atagatagag  | acagacatga  | caattccaaa  | gctgtgtctt  | 1200 |
| aggaggaagt  | aatgagggtac | ctatagaggt  | ttactgtaca  | tgagaaacga  | cttacattca  | 1260 |
| aaacttcaaa  | gagctattga  | ataggaagga  | tgttcaattt  | atttcaaatt  | gcttttagata | 1320 |
| acagaattaa  | ttcaagggct  | gagaacattc  | tagccatatt  | gtctcctcag  | agagaccttc  | 1380 |
| cttaacagcc  | tgtcaacata  | ttcttctctc  | atattgtttt  | ctgaaccctt  | taccccggtt  | 1440 |
| tattcttcat  | agctcttctc  | agtgcattga  | tgtattttta  | tgtgggtcatt | tatctgttat  | 1500 |
| cttctctccc  | acaagaattt  | aggttccaca  | aggactaagc  | ctttgtctat  | cttgagcttg  | 1560 |
| cttatatctc  | cagcatccaa  | gaacagcact  | tggtatatat  | ttatgtaatg  | gttataactt  | 1620 |
| ttgaaatgaa  | tgaataagta  | aataaatatt  | aactgtccaa  | aatgaaaata  | tactccctct  | 1680 |
| gtaagtaaca  | aggctcccat  | cactagaggc  | attctaccag  | gagctgagtg  | atttcgtagg  | 1740 |
| aagcatgctg  | cacagcaggt  | cccagcggat  | gctgcagttg  | cttccctattc | cagacttgga  | 1800 |
| agatttctct  | tctgttggtt  | gtatactctt  | gaggctccat  | gcttccctacc | tgtgggttctt | 1860 |
| tctccatgac  | tctgttggtc  | ctcctttttac | ttcctcttct  | gttctttgct  | tttgggtttgg | 1920 |
| cttcgtggat  | attccttggtc | ctttatcaca  | ttacatcttt  | cattcaaaca  | tagtggtttat | 1980 |
| gaaatcagtc  | ctgttatcgc  | ccttaaagaa  | attctgtgtt  | gaagatataa  | ggagaaagtt  | 2040 |
| aaacaaataa  | atthttgcaat | ttagaaaatg  | cttgctgggtg | tcaaagttct  | ttcactaatt  | 2100 |
| ttattgcagt  | ttccactgag  | tagggagcat  | gcaatttcat  | tcatgcatgc  | aacagatgtt  | 2160 |
| tactgaactc  | tttttgtgta  | ggagacactt  | tgctagatgc  | agagcagaat  | gcactgaatc  | 2220 |
| aaacatagac  | tccttgctct  | aggatctttac | ctctcagtg   | agggagacag  | aaacgttaag  | 2280 |
| gctttctagt  | ctctccttgt  | ctccttttga  | tacttatatc  | agaggagact  | gacctgact   | 2340 |
| tgaatgtctt  | gtgacattcc  | tgcaatttag  | aagaacaagg  | gaagactttt  | tcataaatgt  | 2400 |
| ggacaatctc  | tgtgtacacg  | gagtgaggtt  | ttttcccgct  | tttctctgt   | gtttttaatg  | 2460 |
| gactcataaa  | ctcaccagg   | aatctgtgct  | tttaaaaaaa  | aaaaaaaaa   | a           | 2511 |

&lt;210&gt; 34

&lt;211&gt; 1684

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 34

|            |            |            |            |            |            |     |
|------------|------------|------------|------------|------------|------------|-----|
| gacccacgcg | tccgcgcgcg | cccgccccag | ttatcatggc | ggctcccttg | gtcctgggtg | 60  |
| tggtgggtgg | tgtgacagtg | cggggggcct | tgttccgctc | cagtctggcc | gagttcattt | 120 |
| ccgagcgggt | ggaggtgggt | tccccactga | gctcttgga  | gagagtgggt | gaaggccttt | 180 |
| cactgttggg | acttgggagt | atctccgtat | tctggagcag | tatttcatgg | aaactccatt | 240 |
| aaataaatat | acctctttca | tttccataat | gactatgctg | aattgggtgt | tatgataact | 300 |
| gatgcactca | ctgctattgc | cctgtatttt | gcaatccagg | acttcaataa | agttgtgttt | 360 |
| aaaaagcaga | aactcctcct | agaactggac | cagtatgcc  | cagatgtggc | cgaactcacc | 420 |
| cggaccccta | tggaaatgcg | ttacatccct | ttgaaagtgg | ccctgttcta | tctcttaaat | 480 |

|             |             |             |             |             |             |      |
|-------------|-------------|-------------|-------------|-------------|-------------|------|
| ccttacacga  | ttttgtcttg  | tggttgccaag | tctacctgtg  | ccatcaacaa  | caccttcatt  | 540  |
| gctttcttca  | ttttgactac  | gataaaaggc  | agtgtcttcc  | tcagtgtctat | ttttcttgcc  | 600  |
| ttagcgacat  | accagtctct  | gtaccactc   | accttggttg  | tcccaggact  | cctctatctc  | 660  |
| ctccagcggc  | agtacatacc  | tgtgaaaatg  | aagagcaaaag | ccttctggat  | cttttcttgg  | 720  |
| gagtatgcca  | tgatgtatgt  | gggaagccta  | gtggtaataca | tttgccctctc | cttcttctct  | 780  |
| ctcagctctt  | gggatttcat  | ccccgcagtc  | tatggcttta  | tactttctgt  | tccagatctc  | 840  |
| actccaaaca  | ttgggtctttt | ctgggtacttc | tttgagaga   | tgtttgagca  | cttcagcctc  | 900  |
| ttctttgtat  | gtgtgtttca  | gatcaacgtc  | ttcttctaca  | ccatccccctt | agccataaaag | 960  |
| ctaaaggagc  | acccccatctt | cttcatgttt  | atccagatcg  | ctgtcatcgc  | catctttaag  | 1020 |
| tcctacccga  | cagtggggga  | cgtggcgctc  | tacatggcct  | tcttccccgt  | gtggaaccat  | 1080 |
| ctctacagat  | tcctgagaaa  | catctttgtc  | ctcacctgca  | tcacatcgt   | ctgttccctg  | 1140 |
| ctcttccctg  | tcctgtggca  | cctctggatt  | tatccaggaa  | tgccaactct  | aatttctttt  | 1200 |
| atgccatcac  | actgacctc   | aacgttgggc  | agatcctgct  | catctctgat  | tacttctatg  | 1260 |
| ccttctctgc  | gcgggagtac  | tacctcacac  | atggcctcta  | cttgaccgcc  | aaggatggca  | 1320 |
| cagaggccat  | gctcgtgctc  | aagtaggcct  | ggctggcaca  | gggctgcatg  | gacctcaggg  | 1380 |
| ggctgtgggg  | ccagaagctg  | ggccaagccc  | tccagccaga  | gttgccagca  | ggcgagtgt   | 1440 |
| tgggcgagaag | aggttcgagt  | ccagggtcac  | aagtctctgg  | tacccaaaagg | gacctatggc  | 1500 |
| tgactgacag  | caaggcctat  | ggggaagaac  | tgggagctcc  | ccaacttgga  | ccccacctt   | 1560 |
| gtggctctgc  | acaccaagga  | gccccctccc  | agacaggaag  | gagaagaggc  | aggtgagcag  | 1620 |
| ggcttggttag | attgtggcta  | cttaataaat  | gttttttgtt  | atgaagtcta  | aaaaaaaaaa  | 1680 |
| aaaa        |             |             |             |             |             | 1684 |

&lt;210&gt; 35

&lt;211&gt; 2383

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (538)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 35

|             |             |            |            |             |             |      |
|-------------|-------------|------------|------------|-------------|-------------|------|
| gagcccacga  | gaggcagcgc  | catggcggag | cagacctact | cgtgggccta  | ttccctgggtg | 60   |
| gattccagtc  | aagtgtctac  | atttctgatt | tccattcttc | ttatagtcta  | tggtagtatty | 120  |
| aggtccctta  | atatggactt  | tgaaaatcaa | gataaggaga | aagacagtaa  | tagttcttct  | 180  |
| gggtctttca  | atggcaacag  | caccaataat | agcatccaaa | caattgactc  | taccagggt   | 240  |
| ctgttccctc  | caattggagc  | atctgtctct | cttttagtaa | tgttcttctt  | ctttgactca  | 300  |
| gttcaagtag  | tttttacaat  | atgtacagca | gttcttgcaa | cgatagcttt  | tgcttttctt  | 360  |
| ctcctcccgga | tgtgccagta  | tttaacaaga | cctgtctcac | ctcagaacaa  | gatttccctt  | 420  |
| ggttgctgtg  | gacgtttcac  | tgctgctgag | ttgctgtcat | tctctctgtc  | tgtcatgtc   | 480  |
| gtcctcatct  | gggttctcac  | tggccattgg | cttctcatgg | atgcactggc  | catgggcntc  | 540  |
| tgtgtcgcca  | tgatcgctt   | tgtccgcctg | ccgagcctca | aggtctcctg  | cctgtctctc  | 600  |
| tcagggtctc  | tcacttatga  | tgtcttttgg | gtatttttct | cagcctacat  | cttcaatagc  | 660  |
| aacgtcatgg  | tgaagggtggc | cactcagccg | gctgacaatc | cccttgacgt  | tctatcccg   | 720  |
| aagctccacc  | tggggcccaa  | tggtgggcgt | gatgttcttc | gcctgtctct  | gcctggaaaa  | 780  |
| ctgggtcttc  | caagctccac  | tggcagccac | ttctccatgt | tgggcacggg  | agacatcgtt  | 840  |
| atgcctgggtc | tctactatg   | ctttgtcctt | cgctatgaca | actacaaaaa  | gcaagccagt  | 900  |
| ggggactcct  | gtggggcccc  | tggacctgce | aacatctccg | ggcgcatgca  | gaaggctctc  | 960  |
| tacttttact  | gcacctcat   | cggatacttt | gtaggcctgc | tcactgtctac | tgtggcgctc  | 1020 |
| cgcattccacc | gggcccgcga  | gcccgcctt  | ctctatttgg | tgccatttac  | tttattgcca  | 1080 |
| ctcctcaoga  | tggcctattt  | aaagggcgac | ctccggcgga | tgtggtctga  | gcctttccac  | 1140 |
| tccaagtcca  | gcagctcccc  | attcctggaa | gtatgatgga | tcacgtggaa  | agtgaccaga  | 1200 |
| tggccgtcat  | agtccttttc  | tctcaactca | tggtttgttt | cctcttagag  | ctggcctggt  | 1260 |
| actcagaaat  | gtacctgtgt  | ttaaggaact | gccgtgtgac | tggatttggc  | atttaaagg   | 1320 |
| agctcgtttg  | caggagagag  | gtgctggagc | cctgtttggt | tccttctctt  | cctgcggtatg | 1380 |
| tagagggtggg | gccccctcca  | agagggacag | gcctctcccc | agcgcgccct  | cctcccacgt  | 1440 |
| ttttatggat  | ctgcaccaga  | ctgttacctt | ctgggggaga | tggagatttg  | actgttttaa  | 1500 |

|  |      |
|--|------|
| aactgaaaac agcgaggagt ctttctagaa cttttgaaca ctaaaaggat gaaaaaaatt  | 1560 |
| agcaaaccga agttttcttca atgacccctc gagaactttg ggaccagttt cctatrgggg | 1620 |
| actcagtttc agagaactga gacagaagct cttctgtcgt tatattcttc ttctctttt   | 1680 |
| ttggatttat taaatatttt ctgtggtgtg aagtgactta ttaaattccac agacattgag | 1740 |
| tgactttctta caacatccac ataagrattt gttgtaatga gttcatgtcc acccagatgt | 1800 |
| tgtgttgga gtgaacaagg gcacggtttt tatacatacg tacatatata tatataaaca   | 1860 |
| cacacataga tatatatgaa taaacaaaaa tgaaatcctg ctaagatcac gctgtgtagc  | 1920 |
| tgacaggggc ttgctgtcgt tttgagcatg tcgagcagtt tactgtggct tccttgtata  | 1980 |
| tggataagct gctgtccttc cccttcacaa ctgaccccg agttacaaac tagtatagca   | 2040 |
| tttgtgtga ttgatgatag actcatggac ttcaggagcc ctacttggt ttgatcagt     | 2100 |
| gtagcaaatt agggatgaag agttcaaacc ttttggccct ttctttcttt tctaggcttc  | 2160 |
| tccttcgcag ggtgttcctg agtttcttct cgagccaatg catgtattat agcagcaggt  | 2220 |
| gtctttgtgc tttctcatca tagtaacgta ctacttgtaa atacattttt ctattttcta  | 2280 |
| tttttttgta ttttttttg acattttgtt tcattgggtg gctgtatatt ttccatgccc   | 2340 |
| tcactccttt aagaaaaaaa aaaaaaagg aaaaaagcaa cac                     | 2383 |

&lt;210&gt; 36

&lt;211&gt; 1815

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 36

|  |      |
|--|------|
| cacgcgtccg cggacgctgg gctcaatctc ctgacctgtg gatctgcccg cctcggcctc  | 60   |
| ccaaagtgt ggggtttacag gcatgagcca cagcgcccgg ctgagtattg ggtcttttagg | 120  |
| ggtcaaaact tttgatcttt gcttgacgtt tttgtttttt tctcttttac actctccctg  | 180  |
| ttccctgatt aaatgaaggc caggcttgcc tagttccagg gaaaaggccc aggggtgcta  | 240  |
| gagcaagggt gatgggactt tgttcgcaga tgggccttga gagagcgacc cctcgtcct   | 300  |
| aaatgcccgg aggaaggagc ggacttcttt atctttacca tgggtattct gccttactgc  | 360  |
| tttggcctgt ggcgtttctt cacttgcttt tctcatttt gcttggaaatg tgctttgcct  | 420  |
| gttgcatacc cacctcgtct gccccttgcc acactccatg gctggcctaa aagcccagtc  | 480  |
| tgctgtcctg tgccctttag acttccactg taggattatg ttccacact ccctgtggac   | 540  |
| tgtgccactg gagctctctg cagacaggga ctgtgtcagg ttgacctcca tccttcagac  | 600  |
| cagcccagtg cctggcaggt agaggaaaaga gaagctgagg aaggacttgc tgcacaagtg | 660  |
| gatgccagga gctctggtct tcccttcttg aatctgtctac cttatgatgg gagggacaca | 720  |
| gggctgtgct ggatttgtgc acgatgcttt ggacagccca tgggagaggg ccaggaggaa  | 780  |
| ggaaaccag actgagtga tagcaggctg gatgggggca ttgacagtgg gggaagcatt    | 840  |
| aaaggccatt tatagccttc acaggctctg gtaatgggct cttacacggg ttggtggcgg  | 900  |
| aaggacacag gtggacctgg gctggtggtc actcctgggc tgctcttggc cctggcatct  | 960  |
| gagacctgtt ggccaaaggc tttgatgtgg ctctggtatt tttcttttt tttgagaatg   | 1020 |
| gaactttttt ttttttaatg aaatgctctt ttgaataggg aatacagtca cgtttctaaa  | 1080 |
| atgaaaaat attaaaaat attttaagaa attttgcccc tcactcctga tctcatctct    | 1140 |
| gtcctccctc ctccctggta accacctgta gcagtttgaa tacccttcta gttttctta   | 1200 |
| atgcaagtac agcaaacaca aattgtgtat tattatttct cctttttcag taaatgaaag  | 1260 |
| atagcattct gtgtgtactg ttcttcatct tgtgcttttt ttaacttatt gtagagattt  | 1320 |
| ttccatatca gtgcatggag aatgggtgtc attctctttc agctgtgttg cactgtgaag  | 1380 |
| ttgtccctgt ttgaatactc accctgagg aaaggcacct ggctgtttcc agctgttttc   | 1440 |
| atgacatgcc ggcgacagtt gtctcacgtg cacatcggtt ccacattgc agtggctctg   | 1500 |
| caggggtggca tcccgcaggc acattgctga gtcaaagagg aaacacagtt gtaattttga | 1560 |
| cagattttgc ccagttgccc tctacagggc ttgttccatg ttgactccc actggcgggtg  | 1620 |
| ttgatgcctg attccccact gactcgtcaa cacaaggtgt agtcaaagtc ttggagttct  | 1680 |
| gccagcctga ccaacatgga gaaaccctac tgaggatata aagtttagcca ggcattggtg | 1740 |
| tgcatgcctg tagtcccagc tgctcaggag cctggcaaca agagcaaaac tccagctcaa  | 1800 |
| aaaaaaaaa aaaaa  | 1815 |

&lt;210&gt; 37

&lt;211&gt; 1466

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 37

|  |      |
|--|------|
| acctgcaggt accggtccgg aattcccggg tcgacccacg cgtccgagcc gtccgcgctc  | 60   |
| cgaacggcgc ctccccgccc accatgggca acagcgcgag ccgcaacgac ttcgagtggg  | 120  |
| tctacaccga ccagccgcac acgcagcggc gcgcgcggcc ccctgccaaag taccggcca  | 180  |
| tcaaggccct gatgcccga gaccgcgcgc tcaagtgggc ggtgctggtg ctggtgctgg   | 240  |
| tgcatgct ggctgctgg ctggtgcgcg ggctggcctg gcgctggctg ctgttctggg     | 300  |
| cctacgcctt tgggtggctgc gtgaaccact cgtgacgct ggccatccac gacatctcg   | 360  |
| acaacgcggc cttcggcacg ggccgtgcgg cacgcaaccg ctggctggcc gtgttcgcca  | 420  |
| acctgcccggt ggggtgtgccc tacgcgcct ccttcaagaa gtaccacgtg gaccaccacc | 480  |
| gctacctggg cggcgacggg ctggacgtg acgtgccac gcgtctggag ggctggttct    | 540  |
| tctgcacgcc cgccgcaag ctgctctggc tgggtgctgca gcccttcttc tactactac   | 600  |
| ggccgctctg cgtccacccc aaggccgtga cccgcattga ggtgctcaac acgctggtgc  | 660  |
| agctggcggc cgacctggcc atctttgccc tttgggggct caagcccgtg gtctacctgc  | 720  |
| tggccagctc cttcctgggc ctgggcctgc acccatctc gggccacttc gtggccgagc   | 780  |
| actacatggt cctcaagggc cagagacct actctacta tgggcctctc aactggatca    | 840  |
| ccttcaatgt gggctaccac gtggagcacc acgacttccc cagcatcccg ggctacaacc  | 900  |
| tgccgctggt gcggaagatc gcgcccagat actacgacca cctgccgag caccactcct   | 960  |
| gggtgaaggt gctctgggat tttgtgtttg aggactccct ggggccctat gccagggtga  | 1020 |
| agcgggtgta caggctggca aaagatggtc tgtgagcccg ggtgcctcc tgggtggtggc  | 1080 |
| cattgtcccc catcgcccc tcagccttgc accccagcac tgagaagcta catttccttc   | 1140 |
| ctgtgctctg gactgctgcc cttgtccccg aggagtgtcc cgcgcagcca cacctggcaa  | 1200 |
| cagcagtgtg ggctgcaggg ctccgtctgc acgtggactt gccctggacc ttgagtgtgg  | 1260 |
| ccctcccttt ctgggcctcc ccaggtgagg cctggccctg cccaccatg acctgggtgc   | 1320 |
| tctgagccca cggttccac ggagctgact tctccggggt gcctgtgccc tacattaaac   | 1380 |
| ccggcgttt tttcacaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa   | 1440 |
| aaaaaaaaa aaaaaaaaaa aaaaaa  | 1466 |

&lt;210&gt; 38

&lt;211&gt; 1126

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (206)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1059)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1077)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 38

|  |     |
|--|-----|
| ctgcaggaat tcggcacgag gaactagggt ttcagtttct ccacatcctt gccaacactt  | 60  |
| gttattttct gggatttttt gataatagcc ttcctcatgg atatgaagtg gtttctcatt  | 120 |
| gtagttttga tttgcattcc cctaattgact agtgatattg agcatctttt catgtgccta | 180 |
| ttgccttttc atgtatcttc tttggnsaaa tgtctattca agtcctttgc tcatttttca  | 240 |
| gttggtttgt attttgttgt tgagtttttag ttctctacat attctggata ttaaatoctt | 300 |
| attaaagata tgatttgcaa atattttctc coactctatg ggttgctttt ttactctggt  | 360 |
| gactgtgtct tttgatgcac aaaatttttt aattttcatg aagtctaatt ttctatttct  | 420 |
| cttttgttgc catttccaag aaatcactgc caaatccaat gttgtgaaat tttgccattt  | 480 |

|   |      |
|---|------|
| ttttccctaa tagttttatt gcttttaggt ttacatttag gtcttttcac catttgaggt | 540  |
| tcattttttt acatgacatt aggtaagggt ccaaccacat tattctgcat gtggatatcc | 600  |
| agttttccca gactacttg ttgttgaaaa ggactgtaag gtcttggaac ccttatcaaa  | 660  |
| agctatttga gactagatgt ggtggtatgt gcatgtaatt ccagcttcct gggaagctga | 720  |
| ggcaggaaga ttgctggagc ccaggagttc aaaaccagcc tggacaacct aaggagacct | 780  |
| tetcaaaaaa aaaaagaaga aagaaagaaa aagaaaaggt gaccaggcac agcggstcat | 840  |
| tcccctgkaa tcccagcact ttgggagggt gaggcaggcg gatcagttga ggccaggagt | 900  |
| tcaagaccag cctggscaac ataaccaaac cccaaccca tctctactaa aaatacaaaa  | 960  |
| attagctgga tatggtggca tgcacctgta ttccagcta cttgggagac tgaggcacia  | 1020 |
| gaatcacttg aacctgggag gcagaggttg cagtgagcna aaatcgggcc actgcantcg | 1080 |
| agcctgggca acagagcaag actccaactc aaaaaaaaaa aaaaaa                | 1126 |

&lt;210&gt; 39

&lt;211&gt; 2558

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 39

|   |      |
|---|------|
| ggcagagct gggattacag gcgtgagcca ccgcgccag cctaaagtgt tgatttttat     | 60   |
| ggcaaatggt ccttctctet taatggcccg gggggagatc tgtgtgatag ctggtgttag   | 120  |
| gaaaggctctg agatggagac attcagtgaac gtcagcttcc tgaatgcca cctctctgtg  | 180  |
| ctgtttcttg ctggcactgg gatgaggaca tggctctggt gtggctgtgt tttctcaatt   | 240  |
| ctgttgaggg atttggggtg agtcgtgctc cctctctgct tcctcctttg gaagagaatg   | 300  |
| cccaggacag tgggtgccagt ttcaggtaaa gaaaaacaaa aatagcgctc ttttggactc  | 360  |
| agttctctgt cacttccagt ttgtgagggg acaggtattt gactcagctt ggcaaagcca   | 420  |
| ggaatggagt gttgtagtaa gagcttagca gcgtgctttg ggctttgagc tctgaactta   | 480  |
| agaggagacc tcagttcagt ctacacaggc ttcttacctc aggtgagtca ctttgctggg   | 540  |
| gtctctgggc cagagggtcaa ggctgcccct atgagggctt tggactctga ggggtgtggcg | 600  |
| ttatgtgtgt attagcaaaa tgctgggtgtg cctgggagct ttggggcaat gctcaccagc  | 660  |
| tcttgagttc tggggagtga agtttgggac tggatgctc ttgcattctg agcctctcac    | 720  |
| tcctgtgtct ttcctcttgc ccttcagatg tttgtgaca gccatttctc tcccgaactg    | 780  |
| tctgccagcc cagactgacc tgtctgcttc tctgtctcag cggccagagc acctgtctg    | 840  |
| tttatgcctg gggccgggtt taactgggtcc cccagtccca tgtcacacac acctgttccc  | 900  |
| tttggttag ctgcagtgtc acctctcgtc cctgtttcct tcctctctat gaggtgaaa     | 960  |
| gtcaatgtta cttggagata cagcactcca cagcctacag tcattgtctc tttaatcttt   | 1020 |
| gcagggagtg cgtgaggtca gtaggtaggc attatcgtgc tcattttaca aatggaggac   | 1080 |
| ctgcagccca gagaggtgtg atttgccaa agacacacag ctgctctgct attgagcaca    | 1140 |
| gccagtcctg gaatcaaggt cctccagcat ttggtccatca ttccctgtga aatgttagtg  | 1200 |
| caggctgggc ttggtggctc agggccgtaa ttccagcact ttccgggggct gagacgggag  | 1260 |
| gattgggggtc aggagttcaa gaccagcctg ggcagcatag caagatccta gctttacaaa  | 1320 |
| aacaacaat aacaacaaac aaaatggtaa ttcataggca tgagttaacc acatatgggg    | 1380 |
| tggggatgcc ctagcctcac aatggcccta tgagggttt ggactctgaa agcgaagtca    | 1440 |
| ggtgcggtgt tgtgtgtacc tgtggtccca gctgctcagg atgctgaggc tgaggcagga   | 1500 |
| ggccaagact gcagtgaacc atgatactag cactgcactc cagcctgggc tggagtaaga   | 1560 |
| ccttgctca aggaggggtg gggcttgaaa gcggggcctg ggccccacct cctcacatcc    | 1620 |
| ccagtccctg atagcaccat catgtgggag atgcctgtgt aaccccaagc tgtatcataa   | 1680 |
| agctggacaa caaagacagc tccctggggt ccactgtaat ggaccaaggt ctgtgcgtcc   | 1740 |
| ttcctttcca catcccccta aagttgacaa cagtagtaca tggcacgatg gatgtgtct    | 1800 |
| tggcagggag cttataactc agcttaggcc agatccagga gggcaggtca gtccttgagg   | 1860 |
| atgggcagca gggaccgagg agggcccttg gcgtcccccac accttcgctt ttctacccc   | 1920 |
| ggggataagg ctgagaggcg gtgcccagga aaggccact ttgggcctac tcacagcagc    | 1980 |
| gatattgcca ctttccagag gtggtgaact gattccctag caaccagggg cctgcctga    | 2040 |
| gtaggtcaca gcatgtcatc ggctcctcag gaaagaggaa gactgctcag ctggagctcg   | 2100 |
| ggaccatgaa ggcaggtgtg tgctgggctc tcaggcactg tccctcccag cccacctccc   | 2160 |
| agggctgaac cttagtgaac gggaaaagca ggggtgcagga cagcgatgct tcccaaagcc  | 2220 |
| acctgcaggg ttggtgctgg aagttaccag atggcaccag gctggccgtg ggctcacaat   | 2280 |
| gacctctctt cctagctgga acttccaggg tccctgttgg aaaggaagtg tccagaggac   | 2340 |
| acagcagctg agcaaggggc ccacctctgg ttccttccat cttttactct gcatttctat   | 2400 |

accttcactc tggagggctg ggtgggagga catggcttcc ttttagtctt ctggtagaag 2460  
catggatgca aacaccttcc ttgagtttcc atgagcatag ctgccacagg ctgaccttcc 2520  
caaaagacat tactaaatat aaaaaaaaaa aaaaaaaa 2558

<210> 40  
<211> 1939  
<212> DNA  
<213> Homo sapiens

<400> 40  
cctgttttcc ctctcccttc tatgttgaat ttctgtctat ctaatagcct cctcttaacc 60  
atagtgtcaa tagttttgct tttcttggtt ttagttacct gtggtacagt acaagaagat 120  
gagagagaaa gagagagaga ccattcatgt aactttttatt atagtatatt gtaactgttc 180  
tattttttat tattgtaatt tcttactgtt cctaatttat aaattaaact ttatcacagg 240  
tatgtatgta taggaagaaa catagtgtat atagggtttg gcattataag tagtttcaga 300  
catccactgg gggctcttgg atggatcccc tgcggataag gggggactac agtctttttg 360  
aaaatccctt agcttgtatt gccaacatga tatgtaaact aagaacagac ttaccttgag 420  
agtagacctt agatattcta ctacaagtta agtgcattgg ggggtgtgaat ttctctctcc 480  
tgctctgaa cctttttttt ttcttttgag aagcaagtgc aggggagagg gtacagtggc 540  
ttactctacc ctgttgtttg actatgtgtt gacgtagtgt tttccaagat attcttcatg 600  
tattcccaga gttttgcaga gcctaccagg aactactatg taacacagta tgagaggcga 660  
atcaggactt tagggtagct tatcttacct caggcagagc atttctgcat tcatatgttt 720  
tactaaaagt tttgcaggtc tggttttgtt tgagcaaaga gtttctttgc ttaaaaaaca 780  
aaactttaaa actactatct agataattgg acttgttggc tgagtttttg atatgtgatg 840  
ttaattatac agttaaacca taagcacttt aaggttttat aaaaatatat ttgccttaag 900  
ccatttctaa aatatatttt ccaattgagg gatttaaatg ctttgatttg atcaattact 960  
ttaaattgat gctttaaaaa cacatttttag aacacagcaa tccaaaattt agagcatcaa 1020  
ttacacaaga agtaaaattg tacaaaatta agggcttatt tacatctaag taagtagtgt 1080  
atgtaataaa atagcataat aaaaaggctc tgagtattat gatggaggca tatcatttgg 1140  
tccacaatat aggggaatagg tcttttttaa aactaacaac ttaattctta tcagaaattg 1200  
cctgttttaa tgaaaggtaa ataatttgaa aagaaaaatg taataaataa gagggtagt 1260  
ttttgagaat ataaaagttt aaatatttaa gtgtttataa atattgctat aaatgcattg 1320  
gtgatccagg gctgagctca tctggaattg ggaggtagac caatttctct atatcccatg 1380  
aatggcttgg gcaactcctt gaccattggg tatacagaat gtttaattgat gttgtttgtg 1440  
tgtctcttgt agttctcttg acctacaaag aatcaagggt tagaagttat atatttgaag 1500  
ggctgggttt gtggaaaaat gatcagtgtt ttataaatct aggaattatg aatataattt 1560  
agagaaaaggc aaatttcagt acaataatga gaactgacca aaaatggatt tcaaatagaa 1620  
ctgttctaaa aagggaattg gggccgggtg cgggtggtca tgcctgtaat ctccagcact 1680  
tgaggaggct aggtggggcag atcacctgag gatgggagtt caagatcagc gttaccaaca 1740  
tagagaaacc ccgtctctgc taaatatgca aagttagttc ggcgtggcgg ctcatgcctg 1800  
taatcccagc tactcgggag cctgaggcag gagaatcgct tgaaccaggg aggtggaggt 1860  
tgcgtgagtc gagatcgtgc cattgcactc cagcctgggc aacaagagtg aaactctgtc 1920  
tcaaaaaaaaa aaaaaaaaaa 1939

<210> 41  
<211> 1229  
<212> DNA  
<213> Homo sapiens

<400> 41  
ggcacgaggt ggggtcctcc tctggcctcc tgccctcct gctgctgctg ctgcttccat 60  
tgctggcagc ccagggtggg ggtggcctgc aggcagcgt gctggccctt gaggtggggc 120  
tggtgggtct gggggcctcc tacctgctcc tttgtacagc cctgcacctg cctccagtc 180  
tttctctact cctggcccag ggtaccgcac tggggggcgt cctgggcctg agctggcgcc 240  
gaggcctcat ggggtgttcc ctgggccttg gagctgcctg gctcttagct tggccaggcc 300  
tagctctacc tctggtggct atggcagcgg ggggcagatg ggtgcggcag cagggccccc 360  
gggtgcgcgg gggcatatct cgactctggt tgcgggttct gctgcgcctg taccatgg 420

|            |            |             |             |             |            |      |
|------------|------------|-------------|-------------|-------------|------------|------|
| ccttcggggc | cctgcagggc | tgtggggctg  | tgggggaccg  | gggtctgttt  | gcactgtacc | 480  |
| ccaaaaccaa | caaggatggc | ttccgcagcc  | gcctgcccgt  | ccctggggccc | cgggggcgta | 540  |
| atccccgcac | cacccaacac | ccattagctc  | tgttggcaag  | ggctctgggtc | ctgtgcaagg | 600  |
| gctggaactg | gcgtctggca | cgggccagcc  | agggttttagc | atcccacttg  | cccccgtagg | 660  |
| ccatccacac | actggccagc | tggggcctgc  | ttcgggggtga | acggcccacc  | cgaatcccc  | 720  |
| ggctactacc | acgcagccag | cgcagctag   | ggccccctgc  | ctcccgcag   | ccactgccag | 780  |
| ggactctagc | cgggcggagg | tcacgcaccc  | gccagtccc   | ggccctgccc  | ccctggaggt | 840  |
| agctgactcc | agcccttcca | gccccaaatct | agagcattga  | gcactttatc  | tcccacgact | 900  |
| cagtgaagtt | tctccagtc  | ctagtccctct | cttttcaccc  | accttccctca | gtttgctcac | 960  |
| ttaccccagg | cccagccctt | cggacctcta  | gacaggcagc  | ctcctcagct  | gtggagtcca | 1020 |
| gcagtacttc | tgtgtttctc | tggcgctcct  | cccctaagtt  | attgctgttc  | gcccgtgtg  | 1080 |
| tgtgctcatc | ctcaccctca | ttgactcagg  | cctggggcca  | ggggtgggtg  | aggggtggga | 1140 |
| gagtcagtgt | ttttttctcc | tctttgattt  | tgtttttctg  | tctcccttcc  | aacctgtccc | 1200 |
| ttccccccac | caaaaaaaaa | aaaaaaaaaa  |             |             |            | 1229 |

&lt;210&gt; 42

&lt;211&gt; 1897

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (35)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (406)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 42

ccaccgacgg tggacggacc gctrcragc aactnagtgg atccccggg ctgcaggaat  
60  
tcggcacgag cctccctgca actaagtttt tcttggggaa aaagcctgca gaccaggaag  
120  
tgggaggtgt ctggtgggac actgcctca aaaggagcct ttctgagtgc tggggcttca  
180  
tctgccaggg ctgcacctca gcaggcagtg ggctcccaga gctggctgca gtaacccgca  
240  
ggctgtgccg gcaggatgtg gtcttttggt tctgtttctg ttttagttct gacttgtgcc  
300  
gtggatgtgg ctgagggcct aggggtggga gaagtctcca cagggtgggat tgaactcccc  
360  
aggcacatgg tcttgtggt gctggtggag agagagtccc agaggnttcg gacatgtccc  
420  
gttaaaacct tttcctcaag gtgataggta cgtgtcaagg tgaccctgt ccgccatccg  
480  
gcaggctgga agaagccggt aagatggaca gatcaggag gggtggaggg tcccgtttgt  
540  
gaaggagctg gggaggggga ggcaaggctg agcacagcag cccactgtga tgctctcagt  
600  
gcagaggccc cggggggaga cccagggggg cccccccaa aaccacacca tacagtgagt  
660  
ttcagttccc ccaactcctgc cctttcactt ctctcggtgc gcctggctgc ctcacaggtt  
720  
gtcagcttga gcaaggcccc aagcggcctc tactcagaga cctgggtggt cagaggggag  
780



tggggaaggt ggacatgggc atcagcacag accagcacct ctggtccatc ctgcctggtc  
840  
atccacaggg gagtgccagc tcccaccccc agccacatac tgktctcage ttctgagtgg  
900  
ctgggctgtc ccagagggcc atgctgggcc tgagtgtgt ccttgggcat atgcagatgg  
960  
tgttgtatcc aggggggtttt cagggaagct ttagcacttt catattaaaa caaaaaatgt  
1020  
tggaatctcc attaaatagt caagtcttag ggatagtagt aaaaacatgg ctgtacatgg  
1080  
ctggccaggc acagtggctc acgcctataa tcctagcact ttggggaggct gaggtgggag  
1140  
ggttggccaa gagcttgaga ccagcctggc caacatagca agaccctct ctacttgatt  
1200  
ttttttttta aagtacatgg caaagggcat tataaagtaa gatttgtatt agcttgtagc  
1260  
tggttactga tggtcatagg aagggcctca accataataa tggtgaaata ataaaatgag  
1320  
atgaataaat attagagagg tagaatagta cgtagaaagg ttttagcacac attttgtttt  
1380  
tctgcacgag tggttgttct ctgctttcaa agtgctgaaa cgaatgtcct acattgtctg  
1440  
ccttccatga gggtctgacc gcccttgaga acagatgtac ccactacagg gtgaaggtga  
1500  
gaatgtaatt tgaagctgaa tgagaatgga gctggattct aaaattatat cccctctcag  
1560  
attcacaagt gttcacacat ttaggcaaga agccctaagt cctaaccctg gacatgtgga  
1620  
ttgtccagga ccatcccaga cttttgtggc tggattaatc agacctgagc aaactggatg  
1680  
caaagaggag ggggtaggtt ccgggaaggg gccctgggtg ccagggtggg atgagtgtct  
1740  
ggtgctgagc ccagagccca ggcccttgca atgtttttct tggatctgct gggggcagtt  
1800  
taagctaata aaagcctcct cttttattcc caggacaca tggaagctgc ctctgtccga  
1860  
attcgatatc aagcttatcg ataccgtcga cctcgag  
1897

<210> 43  
<211> 1796  
<212> DNA  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (417)  
<223> n equals a,t,g, or c

<400> 43  
tcgacccacg cgtccgccag attctggagg cgaagaacgc aaagctgaga acatggacgt  
60  
taatatcgcc ccactccgcg cctgggacga tttcttcccg ggttccgac gctttgcccc  
120  
gccggacttc agggacattt ccaaattggaa caaccgcgta gtgagcaacc tgctctatta  
180  
ccagaccaac tacctgggtg tggctgccat gatgatttcc attgtggggg ttctgagtc  
240

cttcaacatg atcctgggag gaatcgtggt ggtgctggtg ttcacagggt ttgtgtgggc  
300  
agcccacaat aaagacgtcc ttgcgcggat gaagaagcgc taccacacga cgttcggtat  
360  
ggtgggtcatg ttggcgagct atttccttat ctccatgttt ggaggagtca tggctctntgt  
420  
gtttggcatt acttttctt tgctgttgat gtttatccat gcacgttga gacttcggaa  
480  
cctcaagaac aaactggaga ataaaatgga aggatagggt gaagaaggac accgatgggc  
540  
atgtcctgga tgcctagaac agcaggaaga aggcacacac agactcactg actatatcag  
600  
caaagtgaag gaataaacat aacttacctg agctagggtt gcagcagaaa ttgagttgca  
660  
gcttgccctt gtccagacct atgttctgct tgcgtttttg aacaggagg tgcacgtacc  
720  
accaattat ctatggcagc atgcatgtat aggcgaact attatcagct ctgatgtttc  
780  
agagagaaga cctcagaaac cgaaagaaaa ccaccacct cctattgtgt ctgaagtttc  
840  
acgtgtgttt atgaaatcta atgggaaatg gatcacacga tttctttaag ggaattaaaa  
900  
aaaataaaaag aattacggct ttacagcaa caatacgatt atcttatagg aaaaaaaaaa  
960  
tcattgtaaa gtatcaagac aatacgagta aatgaaaagg ctgttaaagt agatgacatc  
1020  
atgtgttagc ctgttcctaa tcccctagaa ttgtaatgtg tgggatataa attagttttt  
1080  
attattctct taaaaatcaa agatgatctc tatcactttg ccacctgttt gatgtgcagt  
1140  
ggaaactggt taagccagtt gttcatactt cttttacaaa tataaagata gctgtttagg  
1200  
atattttgtt acatttttgt aaatttttga aatgctagta atgtgttttc accagcaagt  
1260  
atttgttgca aacttaatgt cattttctt aagatggta cagctatgta acctgtatta  
1320  
ttctggacgg acttattaaa atacaaacag acaaaaaata aaacaaaact tgagttctat  
1380  
ttaccttgca cattttttgt tgttacagtg aaaaaaatgg tccaagaaaa tgtttgccat  
1440  
ttttgcattg tttcgttttt aactggaaca tttagaaaga aggaaatgaa tgtgcatttt  
1500  
attaattcct taggggcaca aggaggacaa taatagctga tcttttgaaa tttgaaaaac  
1560  
gtcttttagat gaccaagcaa aaagacttta aaaaatggta atgaaaatgg aatgcagcta  
1620  
ctgcagctaa taaaaaattt tagatagcaa ttgttacaac catatgcctt tatagctaga  
1680  
cattagaatt atgatagcat gagtttatac attctattat ttttctccc tttctcatgt  
1740  
ttttataaat aggtaataaa aaatgttttg cctgccaaaa aaaaaaaaaa aaaaaa  
1796

&lt;210&gt; 44

&lt;211&gt; 2136

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

<400> 44  
ggggacggag cgcgtgtcaa ctctccaact cagctcagct gatcggttgc cgccgccgcc  
60  
gccgccagat tctggaggcg aagaacgcaa agctgagaac atggacgtta atatcgcccc  
120  
actccgcgcc tgggacgatt tcttcccggg ttccgatcgc tttgcccggc cggacttcag  
180  
ggacatttcc aaatggaaca accgcgtagt gagcaacctg ctctattacc agaccaacta  
240  
cctgggtggtg gctgccatga tgatttccat tgtgggggtt ctgagtcctt tcaacatgat  
300  
cctggggagga atcgtggtgg tgctggtgtt cacagggtt gtgtgggcag cccacaataa  
360  
agacgtcctt cgccggatga agaagcgcta cccacgacg ttcgttatgg tggtcattgt  
420  
ggcgagctat ttccttatct ccatgtttgg aggagtcatg gtctttgtgt ttggcattac  
480  
ttttcctttg ctgttgatgt ttatccatgc atcgttgaga cttcggaacc tcaagaacaa  
540  
actggagaat aaaatggaag gaatagggtt gaagaggaca ccatggggca ttgtcctgga  
600  
tgccctagaa cagcaggaag aaggcatcaa cagactcact gactatatca gcaaagtga  
660  
ggaataaaca taacttacct gagctagggt tgcagcagaa attgagttgc agcttgccct  
720  
tgtccagacc tatktttctgc ttgcgttttt gaaacaggag gtgcacgtac caccacaatta  
780  
tctatggcag catgcatgta taggccaac tattatcagc tctgatgttt cagagagaag  
840  
acctcagaaa ccgaaagaaa accaccaccc tctattgtg tctgaagttt cacgtgtgtt  
900  
tatgaaatct aatgggaaat ggatcacacg atttctttta ggggaattaaa aaaaataaaa  
960  
gaattacggc ttttacagca acaatacgat tatcttatag gaaaaaaaa atcattgtaa  
1020  
agtatcaaga caatacgagt aaatgaaaag gctgttaaag tagatgacat catgtgttag  
1080  
cctgttccta atcccctaga attgtaatgt gtgggatata aattagtttt tattattctc  
1140  
ttaaaaatca aagatgatct ctatcacttt gccacctgtt tgatgtgcag tggaaactgg  
1200  
ttaagccagt tgttcatact tcstttacaa atataaagat agctgttttag gatattttgt  
1260  
tacatttttg taaatttttg aaatgctagt aatgtgtttt caccagcaag tatttgttgc  
1320  
aaacttaatg tcatttttct taagatgggt acagctatgt aacctgtatt attctggacg  
1380  
gacttattaa aatacaaaca gacaaaaaat aaaacaaaac ttgagttcta tttaccttgc  
1440  
acattttttg ttgttacagt gaaaaaaatg gtccaagaaa atgtttgcca tttttgcatt  
1500  
gtttcgtttt taactggaac atttagaaag aaggaaatga atgtgcattt tattaattcc  
1560  
ttaggggcac aaggaggaca ataatagctg atcttttgaa atttgaaaaa cgtctttaga  
1620  
tgaccaagca aaaagacttt aaaaaatggg aatgaaaatg gaatgcagct actgcagcta  
1680  
ataaaaaatt ttagatagca attgttacia ccatatgcct ttatagctag acattagaat  
1740

tatgatagca tgagtttata cattctatta tttttcctcc ctttctcatg tttttataaa  
1800  
taggtaataa aaaatgtttt gcctgccaat tgaatgattt cgtagctgaa gtagaaacat  
1860  
ttaggtttct gtagcattaa attgtgaaga caactggagt ggtacttact gaagaaactc  
1920  
tctgtatgtc ctagaataag aagcaatgat gtgctgcttc tgatttttct tgcattttaa  
1980  
attctcagcc aacctacagc catgatcttt agcacagtga tatcaccatg acttcacaga  
2040  
catgggtctag aatctgtacc cttaccacaca tatgaagaat aaaattgatt aaagggttaa  
2100  
aaaaaaawaa aaaaamwagg ggggcccggt wcccag  
2136

<210> 45  
<211> 2081  
<212> DNA  
<213> Homo sapiens

<400> 45  
ttattcggaa ttaacctca ctaaaggcaa caaagctggg agctccaccg cggtggcggc  
60  
cgctctagaa ctagtggatc ccccgggctg caggggaattc ggcacgagct tgtaagaagg  
120  
ctcatgccat tgacctctt aattctctcc tgtttggcgg actggacaat ggcggaggct  
180  
gaaggcaatg caagctgcac agtcagtcta gggggtgcca atatggcaga gaccacaaa  
240  
gccatgatcc tgcaactcaa tcccagttag aactgcacct ggacaataga aagaccagaa  
300  
aacaaaagca tcagaattat cttttcctat gtcccagctt gatccagatg gaagctgtga  
360  
aagtgaaac attaaagtct ttgacggaac ctccagcaat gggcctctgc tagggcaagt  
420  
ctgcagtaaa aacgactatg ttctgtatt tgaatcatca tccagtacat tgacgtttca  
480  
aatagttact gactcagcaa gaattcaaag aactgtcttt gtcttctact acttcttctc  
540  
tcctaacatc tctattccaa actgtggcgg ttacctggat accttgaag gatccttcac  
600  
cagccccaat tacccaaagc cgcctcctga gctggcttat tgtgtgtggc acatacaagt  
660  
ggagaaagat tacaagataa aactaaactt caaagagatt ttccatagaaa tagacaaaca  
720  
gtgcaaattt gattttcttg ccatctatga tggccctcc accaactctg gcctgattgg  
780  
acaagtctgt ggccgtgtga ctcccacctt cgaatcgtca tcaaactctc tgactgtcgt  
840  
gttgtctaca gattatgcca attcttaccg gggattttct gcttctaca cctcaattta  
900  
tgcagaaaac atcaacacta catctttaac ttgctcttct gacaggatga gagttattat  
960  
aagcaaatcc tacctagagg cttttaactc taatgggaat aacttgcaac taaaagacct  
1020  
aacttggcag accaaaatta tcaaatggtg ggggaatttt ctggtcctct taatggatgt  
1080

ggtacattca gaaaggtaga aagttcagtc aattccttcc accaatataa tccctttttc  
1140  
tgcatactca acttctaaag tgatcacccg tcagaaacaa ctccagatta ttgtgaagt  
1200  
taatggacat aattctacag tggagataat atacataaca gaagatgatg taatacaaa  
1260  
tcaaaatgca ctgggcaaata ataacaccag catggctctt tttgaatcca attcatttga  
1320  
aaagactata cttgaatcac catattatgt ggatttgaac caaactcttt ttgttcaagt  
1380  
tagtctgcac acctcagatc caaatttggt ggtgtttctt gatacctgta gagcctctcc  
1440  
cacctctgac tttgcatctc caacctacga cctaatacaag agtggatgta gtcgagatga  
1500  
aacttgtaag gtgtatccct tatttggaca ctatgggaga ttccagttta atgccttta  
1560  
attcttgaga agtatgagct ctgtgtatct gcagtgtaaa gttttgatat gtgatagcag  
1620  
tgaccaccag tctcgtgca atcaaggttg tgtctccaga agcaaacgag acatttcttc  
1680  
atataaatgg aaaacagatt ccatcatagg acccattcgt ctgaaaagg atcgaagtgc  
1740  
aagtggaat tcaggatttc agcatgaaac acatgcggaa gaaactccaa accagccttt  
1800  
caacagtgtg catctgtttt ccttcattgt tctagctctg aatgtggtga ctgtagcgac  
1860  
aatcacagtg aggcattttg taaatcaacg ggcagactac aaataccaga agctgcagaa  
1920  
ctattaacta acaggtccaa ccctaagtga gacatgtttc tccaggatgc caaaggaaat  
1980  
gctacctcgt ggctacacat attatgaata aatgaggaag ggcctgaaag tgacacacag  
2040  
gcctgcaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa a  
2081

<210> 46

<211> 1135

<212> DNA

<213> Homo sapiens

<400> 46

gcttgaccgc ccacctggca ggagcaggac aggacggccg gacgcgccca tggccgagct  
60  
cccgggggccc tttctctgcg gggccctgct aggcttctctg tgcctgagtg ggctggccgt  
120  
ggaggtgaag gtacccacag agccgctgag cagccccctg gggaagacag ccgagctgac  
180  
ctgcacctac agcacgtcgg tgggagacag cttcgccctg gagtggagct ttgtgcagcc  
240  
tgggaaaccc atctctgagt cccatccaat cctgtacttc accaatggcc atctgtatcc  
300  
aactggttct aagtcaaagc gggtcagcct gcttcagaac cccccacag tgggggtggc  
360  
cacactgaaa ctgactgacg tccaccctc agatactgga acctacctct gccaaagtcaa  
420  
caaccaccca gatttctaca ccaatgggtt ggggctaata aaccttactg tgctggttcc  
480

ccccagtaat cccttatgca gtcagagtgg acaaacctct gtgggagggt ctactgcact  
540  
gagatgcagc tcttccgagg gggctcctaa gccagtgtac aactgggtgc gtcttggaaac  
600  
ttttcctaca ccttctcctg gcagcatggt tcaagatgag gtgtctggcc agctcattct  
660  
caccaacctc tccctgacct cctcgggcac ctaccgctgt gtggccacca accagatggg  
720  
cagtgcaccc tgtgagctga cctstctgt gaccgaaccc tcccaaggcc gagtggcgga  
780  
gctctgattg ggggtgctct gggcgtgctg ttgctgtcag ttgctgcgtt ctgctgggtc  
840  
aggttccaga aagagagggg gaagaagccc aaggagacat atgggggtag tgaccttcgg  
900  
gaggatgcca tcgctcctgg gatctctgag cacacttgta tgagggtga ttctagcaag  
960  
gggttctctg aaagacctc gtctgccagc accgtgacga ccaccaagtc caagctccct  
1020  
atggctcgtg gacttctccc gatccctgag ggcgggtgagg gggaatatca ataattaaag  
1080  
tctgtgggta ccawaaaaaa aaaaaaaaaa aaaaaaactc gagggggggc ccggt  
1135

<210> 47  
<211> 1227  
<212> DNA  
<213> Homo sapiens

<400> 47  
ggcacgaggt ggggtcctcc tctggcctcc tgcctctct gctgctgctg ctgcttccat  
60  
tgctggcagc ccagggtggg ggtggcctgc aggcagcgt gctggccctt gaggtggggc  
120  
tggtgggtct gggggcctcc tacctgctcc tttgtacagc cctgcacctg cctccagtc  
180  
tttctctact cctggcccag ggtaccgcac tgggggcccgt cctgggcctg agctggcgcc  
240  
gaggeetcat ggggtgtccc ctgggccttg gagctgctg gctcttagct tggccaggcc  
300  
tagctctacc tctggtggct atggcagcgg ggggcagatg ggtgcggcag caggggcccc  
360  
gggtgcgccg gggcatatct cgactctggt tgcgggttct gctgcgctg tcacccatgg  
420  
ccttccgggc cctgcagggc tgtggggctg tgggggaccg ggtctgttt gactgtacc  
480  
ccaaaaccaa caaggatggc ttccgcagcc gctgcccgt cctggggccc cggcggcgta  
540  
atccccgcac caccacaacac ccattagctc tgttggaag ggtctgggtc ctgtgcaagg  
600  
gctggaactg gcgtctggca cgggccagcc aggggttagc atcccacttg cccccgtggg  
660  
ccatccacac actggccagc tggggcctgc ttgggggtga acggccaccc gaatcccccg  
720  
gctactacca cgcagccagc gccagctagg gcccctgccc tcccgcagc cactgccagg  
780  
gactctagcc gggcggaggt cagcacccg ccagtcctcg gccctgcccc cctggaggta  
840

gctgactcca gcccttccag cccaaatcta gagcattgag cactttatct cccacgactc  
 900  
 agtgaagttt ctccagtcct tagtcctctc ttttcaccca ccttcctcag tttgctcact  
 960  
 taccacaggc ccagccttcg gacctctaga caggcagcct cctcagctgt ggagtccagc  
 1020  
 agtcactctg tggtctcctg gcgctcctcc cctaagttat tgctgttcgc ccgctgtgtg  
 1080  
 tgctcatcct caccctcatt gactcaggcc tggggccagg ggtggtggag ggtgggaaga  
 1140  
 gtcattgtttt ttttctcctc ttgattttg tttttctgtc tcccttccaa cctgtccctt  
 1200  
 cccccacca aaaaaaaaaa aaaaaaa  
 1227

<210> 48  
 <211> 41  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (41)  
 <223> Xaa equals stop translation

<400> 48  
 Met Pro Leu Gln Pro Trp Asp Thr Phe Met Ile Leu Gly Leu Tyr Phe  
 1 5 10 15  
 Leu Val Ser Gly Met Thr Ser Asp Ser Ala Gly Gln Gly Lys Leu Asn  
 20 25 30  
 Ser Val Gln Asp Gly His His Trp Xaa  
 35 40

<210> 49  
 <211> 294  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (294)  
 <223> Xaa equals stop translation

<400> 49  
 Met Val Ile Phe Thr Leu Ser Val Ser Met Leu Leu Arg Tyr Ser His  
 1 5 10 15  
 His Gln Ile Phe Val Phe Ile Ala Pro Leu Leu Thr Val Ile Leu Ala  
 20 25 30  
 Leu Val Gly Met Glu Ala Ile Met Ser Glu Phe Phe Asn Asp Thr Thr  
 35 40 45  
 Thr Ala Phe Tyr Ile Ile Leu Ile Val Trp Leu Ala Asp Gln Tyr Asp  
 50 55 60

Ala Ile Cys Cys His Thr Ser Thr Ser Lys Arg His Trp Leu Arg Phe  
 65 70 75 80  
 Phe Tyr Leu Tyr His Phe Ala Phe Tyr Ala Tyr His Tyr Arg Phe Asn  
 85 90 95  
 Gly Gln Tyr Ser Ser Leu Ala Leu Val Thr Ser Trp Leu Phe Ile Gln  
 100 105 110  
 His Ser Met Ile Tyr Phe Phe His His Tyr Glu Leu Pro Ala Ile Leu  
 115 120 125  
 Gln Gln Val Arg Ile Gln Glu Met Leu Leu Gln Ala Pro Pro Leu Gly  
 130 135 140  
 Pro Gly Thr Pro Thr Ala Leu Pro Asp Asp Met Asn Asn Asn Ser Gly  
 145 150 155 160  
 Ala Pro Ala Thr Ala Pro Asp Ser Ala Gly Gln Pro Pro Ala Leu Gly  
 165 170 175  
 Pro Val Ser Pro Gly Ala Ser Gly Ser Pro Gly Pro Val Ala Ala Ala  
 180 185 190  
 Pro Ser Ser Leu Val Ala Ala Ala Ala Ser Val Ala Ala Ala Ala Gly  
 195 200 205  
 Gly Asp Leu Gly Trp Met Ala Glu Thr Ala Ala Ile Ile Thr Asp Ala  
 210 215 220  
 Ser Phe Leu Ser Gly Leu Ser Ala Ser Leu Leu Glu Arg Arg Pro Ala  
 225 230 235 240  
 Ser Pro Leu Gly Pro Ala Gly Gly Leu Pro His Ala Pro Gln Asp Ser  
 245 250 255  
 Val Pro Pro Ser Asp Ser Ala Ala Ser Asp Thr Thr Pro Leu Gly Ala  
 260 265 270  
 Ala Val Gly Gly Pro Ser Pro Ala Ser Met Ala Pro Thr Glu Ala Pro  
 275 280 285  
 Ser Glu Val Gly Ser Xaa  
 290

<210> 50  
 <211> 119  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (119)  
 <223> Xaa equals stop translation

<400> 50  
 Met Ala Gly Pro Arg Gly Leu Leu Pro Leu Cys Leu Leu Ala Phe Cys



1

5

34  
10

15

Leu Ala Gly Phe Ser Phe Val Arg Gly Gln Val Leu Phe Lys Gly Cys  
20 25 30

Asp Val Lys Thr Thr Phe Val Thr His Val Pro Cys Thr Ser Cys Ala  
35 40 45

Ala Ile Lys Lys Gln Thr Cys Pro Ser Gly Trp Leu Arg Glu Leu Pro  
50 55 60

Asp Gln Ile Thr Gln Asp Cys Arg Cys Gly Pro Pro Leu Ser Leu Pro  
65 70 75 80

Val Ser Arg Ser Ile Leu Trp Gly Gly Arg Asp Ser Gly Ser Leu Thr  
85 90 95

Gly Pro Gln Asn Glu Glu Lys His Ser Leu Ile His Ala Pro Val Ala  
100 105 110

Pro Pro Gly Trp Trp Arg Xaa  
115

&lt;210&gt; 51

&lt;211&gt; 77

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (77)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 51

Met Thr Ser Ile Phe Thr Ser Leu Ala Val Val Thr Gly Val Leu Ile  
1 5 10 15

Leu Val Gly Cys Cys Ile Thr Pro Ser Val His Gly Leu Val Gln Arg  
20 25 30

Leu Thr Glu Thr Ala Leu Thr Lys Thr Ser Leu Asn Ser Ser Pro Pro  
35 40 45

Tyr Ser Asp Lys Leu Pro Leu Leu Asp His Gln Glu Glu Gln Gln Ser  
50 55 60

Gln Ile Met Phe Glu Lys Phe Glu Glu Gly Lys Leu Xaa  
65 70 75

&lt;210&gt; 52

&lt;211&gt; 70

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (70)

<223> Xaa equals stop translation

<400> 52

Met Trp Ser Leu Val Ser Val Ser Val Leu Val Leu Thr Cys Ala Val  
1 5 10 15

Asp Val Ala Glu Gly Leu Gly Trp Gly Glu Val Ser Thr Gly Gly Ile  
20 25 30

Glu Leu Pro Arg His Met Val Leu Val Val Leu Val Glu Arg Glu Phe  
35 40 45

Pro Glu Val Ser Asp Met Leu Pro Leu Lys Pro Phe Pro Gln Gly Asp  
50 55 60

Arg Tyr Val Ser Arg Xaa  
65 70

<210> 53

<211> 320

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (320)

<223> Xaa equals stop translation

<400> 53

Met Ser Ser Asn Lys Glu Gln Arg Ser Ala Val Phe Val Ile Leu Phe  
1 5 10 15

Ala Leu Ile Thr Ile Leu Ile Leu Tyr Ser Ser Asn Ser Ala Asn Glu  
20 25 30

Val Phe His Tyr Gly Ser Leu Arg Gly Arg Ser Arg Arg Pro Val Asn  
35 40 45

Leu Lys Lys Trp Ser Ile Thr Asp Gly Tyr Val Pro Ile Leu Gly Asn  
50 55 60

Lys Thr Leu Pro Ser Arg Cys His Gln Cys Val Ile Val Ser Ser Ser  
65 70 75 80

Ser His Leu Leu Gly Thr Lys Leu Gly Pro Glu Ile Glu Arg Ala Glu  
85 90 95

Cys Thr Ile Arg Met Asn Asp Ala Pro Thr Thr Gly Tyr Ser Ala Asp  
100 105 110

Val Gly Asn Lys Thr Thr Tyr Arg Val Val Ala His Ser Ser Val Phe  
115 120 125

Arg Val Leu Arg Arg Pro Gln Glu Phe Val Asn Arg Thr Pro Glu Thr  
130 135 140

Val Phe Ile Phe Trp Gly Pro Pro Ser Lys Met Gln Lys Pro Gln Gly  
145 150 155 160

Ser Leu Val Arg Val Ile Gln Arg Ala Gly Leu Val Phe Pro Asn Met  
 165 170 175  
 Glu Ala Tyr Ala Val Ser Pro Gly Arg Met Arg Gln Phe Asp Asp Leu  
 180 185 190  
 Phe Arg Gly Glu Thr Gly Lys Asp Arg Glu Lys Ser His Ser Trp Leu  
 195 200 205  
 Ser Thr Gly Trp Phe Thr Met Val Ile Ala Val Glu Leu Cys Asp His  
 210 215 220  
 Val His Val Tyr Gly Met Val Pro Pro Asn Tyr Cys Ser Gln Arg Pro  
 225 230 235 240  
 Arg Leu Gln Arg Met Pro Tyr His Tyr Tyr Glu Pro Lys Gly Pro Asp  
 245 250 255  
 Glu Cys Val Thr Tyr Ile Gln Asn Glu His Ser Arg Lys Gly Asn His  
 260 265 270  
 His Arg Phe Ile Arg Glu Lys Gly Leu Leu Ile Val Gly Pro Ala Val  
 275 280 285  
 Trp His His Leu Leu Pro Pro Leu Leu Asp Leu Gly His Pro Ala Cys  
 290 295 300  
 Gly Thr Ser Gly Gly Ser Glu Glu Lys Gln Pro Pro Pro Ser Arg Xaa  
 305 310 315 320

<210> 54  
 <211> 97  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (97)  
 <223> Xaa equals stop translation

<400> 54  
 Met Ala Ala Ser Leu Gly Gln Val Leu Ala Leu Val Leu Val Ala Ala  
 1 5 10 15  
 Leu Trp Gly Gly Thr Gln Pro Leu Leu Lys Arg Ala Ser Ala Gly Leu  
 20 25 30  
 Gln Arg Val His Glu Pro Thr Trp Ala Gln Gln Leu Leu Gln Glu Met  
 35 40 45  
 Lys Thr Leu Phe Leu Asn Thr Glu Tyr Leu Met Pro Phe Leu Leu Asn  
 50 55 60  
 Gln Cys Gly Ser Leu Leu Tyr Tyr Leu Thr Leu Ala Ser Thr Gly Trp

65

70

37

75

80

Ser Gln Thr Ser Glu Phe Arg Ser Ser Cys Trp Asn Pro Gly Lys His  
 85 90 95

Xaa

&lt;210&gt; 55

&lt;211&gt; 373

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (81)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (162)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (314)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (315)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (373)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 55

Met Ala Trp Thr Lys Tyr Gln Leu Phe Leu Ala Gly Leu Met Leu Val  
 1 5 10 15

Thr Gly Ser Ile Asn Thr Leu Ser Ala Lys Trp Ala Asp Asn Phe Met  
 20 25 30

Ala Glu Gly Cys Gly Gly Ser Lys Glu His Ser Phe Gln His Pro Phe  
 35 40 45

Leu Gln Ala Val Gly Met Phe Leu Gly Glu Phe Ser Cys Leu Ala Ala  
 50 55 60

Phe Tyr Leu Leu Arg Cys Arg Ala Ala Gly Gln Ser Asp Ser Ser Val  
 65 70 75 80

Xaa Pro Gln Gln Pro Phe Asn Pro Leu Leu Phe Leu Pro Pro Ala Leu  
 85 90 95

Cys Asp Met Thr Gly Thr Ser Leu Met Tyr Val Ala Leu Asn Met Thr

100

38

105

110

Ser Ala Ser Ser Phe Gln Met Leu Arg Gly Ala Ser Asp His Ile His  
115 120 125

Trp Pro Val Leu Gly Gly Leu Pro Gly Pro Glu Ala Gly Ala Glu Pro  
130 135 140

Val Ala Gly His Pro Ser His His Arg Gly Ala Gly Gly Arg Gly Pro  
145 150 155 160

Gly Xaa Pro Pro Glu Gln Ala Arg Gln Ser Ser Thr Ser Phe Ser Glu  
165 170 175

Val Ile Thr Gly Asp Leu Leu Ile Ile Met Ala Gln Ile Ile Val Ala  
180 185 190

Ile Gln Met Val Leu Glu Glu Lys Phe Val Tyr Lys His Asn Val His  
195 200 205

Pro Leu Arg Ala Val Gly Thr Glu Gly Leu Phe Gly Phe Val Ile Leu  
210 215 220

Ser Leu Leu Leu Val Pro Met Tyr Tyr Ile Pro Ala Gly Ser Phe Ser  
225 230 235 240

Gly Asn Pro Arg Gly Thr Leu Glu Asp Ala Leu Asp Ala Phe Cys Gln  
245 250 255

Val Gly Gln Gln Pro Leu Ile Ala Val Ala Leu Leu Gly Asn Ile Ser  
260 265 270

Ser Ile Ala Phe Phe Asn Phe Ala Gly Ile Ser Val Thr Lys Glu Leu  
275 280 285

Ser Ala Thr Thr Arg Met Val Leu Asp Ser Leu Arg Thr Val Val Ile  
290 295 300

Trp Ala Leu Ser Leu Ala Leu Gly Trp Xaa Xaa Phe His Ala Leu Gln  
305 310 315 320

Ile Leu Gly Phe Leu Ile Leu Leu Ile Gly Thr Ala Leu Tyr Asn Gly  
325 330 335

Leu His Arg Pro Leu Leu Gly Arg Leu Ser Arg Gly Arg Pro Leu Ala  
340 345 350

Glu Glu Ser Glu Gln Glu Arg Leu Leu Gly Gly Thr Arg Thr Pro Ile  
355 360 365

Asn Asp Ala Ser Xaa  
370

&lt;210&gt; 56

&lt;211&gt; 491

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 56

Met Glu Asn Glu Glu Ser Asp Val Lys Pro Pro Asp Trp Pro Asn Pro  
 1 5 10 15  
 Met Asn Ala Thr Ser Gln Phe Pro Gln Pro Gln His Phe Asp Ser Phe  
 20 25 30  
 Gly Leu Arg Leu Pro Arg Asp Ile Thr Glu Leu Pro Glu Trp Ser Glu  
 35 40 45  
 Gly Tyr Pro Phe Tyr Met Ala Met Gly Phe Pro Gly Tyr Asp Leu Ser  
 50 55 60  
 Ala Asp Asp Ile Ala Gly Lys Phe Gln Phe Ser Arg Gly Met Arg Arg  
 65 70 75 80  
 Ser Tyr Asp Ala Gly Phe Lys Leu Met Val Val Glu Tyr Ala Glu Ser  
 85 90 95  
 Thr Asn Asn Cys Gln Ala Ala Lys Gln Phe Gly Val Leu Glu Lys Asn  
 100 105 110  
 Val Arg Asp Trp Arg Lys Val Lys Pro Gln Leu Gln Asn Ala His Ala  
 115 120 125  
 Met Arg Arg Ala Phe Arg Gly Pro Lys Asn Gly Arg Phe Ala Leu Val  
 130 135 140  
 Asp Gln Arg Val Ala Glu Tyr Val Arg Tyr Met Gln Ala Lys Gly Asp  
 145 150 155 160  
 Pro Ile Thr Arg Glu Ala Met Gln Leu Lys Ala Leu Glu Ile Ala Gln  
 165 170 175  
 Glu Met Asn Ile Pro Glu Lys Gly Phe Lys Ala Ser Leu Gly Trp Cys  
 180 185 190  
 Arg Arg Met Met Arg Arg Tyr Asp Leu Ser Leu Arg His Lys Val Pro  
 195 200 205  
 Val Pro Gln His Leu Pro Glu Asp Leu Thr Glu Lys Leu Val Thr Tyr  
 210 215 220  
 Gln Arg Ser Val Leu Ala Leu Arg Arg Ala His Asp Tyr Glu Val Ala  
 225 230 235 240  
 Gln Met Gly Asn Ala Asp Glu Thr Pro Ile Cys Leu Glu Val Pro Ser  
 245 250 255  
 Arg Val Thr Val Asp Asn Gln Gly Glu Lys Pro Val Leu Val Lys Thr  
 260 265 270  
 Pro Gly Arg Glu Lys Leu Lys Ile Thr Ala Met Leu Gly Val Leu Ala  
 275 280 285  
 Asp Gly Arg Lys Leu Pro Pro Tyr Ile Ile Leu Arg Gly Thr Tyr Ile  
 290 295 300  
 Pro Pro Gly Lys Phe Pro Ser Gly Met Glu Ile Arg Cys His Arg Tyr

305

40

315

320

Gly Trp Met Thr Glu Asp Leu Met Gln Asp Trp Leu Glu Val Val Trp  
 325 330 335

Arg Arg Arg Thr Gly Ala Val Pro Lys Gln Arg Gly Met Leu Ile Leu  
 340 345 350

Asn Gly Phe Arg Gly His Ala Thr Asp Ser Val Lys Asn Ser Met Glu  
 355 360 365

Ser Met Asn Thr Asp Met Val Ile Ile Pro Gly Gly Leu Thr Ser Gln  
 370 375 380

Leu Gln Val Leu Asp Val Val Val Tyr Lys Pro Leu Asn Asp Ser Val  
 385 390 395 400

Arg Ala Gln Tyr Ser Asn Trp Leu Leu Ala Gly Asn Leu Ala Leu Ser  
 405 410 415

Pro Thr Gly Asn Ala Lys Lys Pro Pro Leu Gly Leu Phe Leu Glu Trp  
 420 425 430

Val Met Val Ala Trp Asn Ser Ile Ser Ser Glu Ser Ile Val Gln Gly  
 435 440 445

Phe Lys Asn Cys His Ile Ser Ser Asn Leu Glu Glu Glu Asp Asp Val  
 450 455 460

Leu Trp Glu Ile Glu Ser Glu Leu Pro Gly Gly Gly Glu Pro Pro Lys  
 465 470 475 480

Asp Cys Asp Thr Glu Ser Met Ala Glu Ser Asn  
 485 490

&lt;210&gt; 57

&lt;211&gt; 188

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 57

Met Asp Val Asn Ile Ala Pro Leu Arg Ala Trp Asp Asp Phe Phe Pro  
 1 5 10 15

Gly Ser Asp Arg Phe Ala Arg Pro Asp Phe Arg Asp Ile Ser Lys Trp  
 20 25 30

Asn Asn Arg Val Val Ser Asn Leu Leu Tyr Tyr Gln Thr Asn Tyr Leu  
 35 40 45

Val Val Ala Ala Met Met Ile Ser Ile Val Gly Phe Leu Ser Pro Phe  
 50 55 60

Asn Met Ile Leu Gly Gly Ile Val Val Val Leu Val Phe Thr Gly Phe  
 65 70 75 80

Val Trp Ala Ala His Asn Lys Asp Val Leu Arg Arg Met Lys Lys Arg  
 85 90 95

Tyr Pro Thr Thr Phe Val Met Val Val Met Leu Ala Ser Tyr Phe Leu  
 100 105 110

Ile Ser Met Phe Gly Gly Val Met Val Phe Val Phe Gly Ile Thr Phe  
 115 120 125

Pro Leu Leu Leu Met Phe Ile His Ala Ser Leu Arg Leu Arg Asn Leu  
 130 135 140

Lys Asn Lys Leu Glu Asn Lys Met Glu Gly Ile Gly Leu Lys Arg Thr  
 145 150 155 160

Pro Met Gly Ile Val Leu Asp Ala Leu Glu Gln Gln Glu Glu Gly Ile  
 165 170 175

Asn Arg Leu Thr Asp Tyr Ile Ser Lys Val Lys Glu  
 180 185

<210> 58

<211> 41

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (41)

<223> Xaa equals stop translation

<400> 58

Met Met Gly Glu Arg Cys Leu Ala Leu Asn Val Leu Phe Ala Gly Val  
 1 5 10 15

Ala Ser Cys Gln Arg Leu Phe Ser Arg Asn Leu Ser Cys His Cys Phe  
 20 25 30

Gly Asp Tyr Cys Asp Pro Ser Leu Xaa  
 35 40

<210> 59

<211> 315

<212> PRT

<213> Homo sapiens

<400> 59

Met Pro Leu Thr Leu Leu Ile Leu Ser Cys Leu Ala Glu Leu Thr Met  
 1 5 10 15

Ala Glu Ala Glu Gly Asn Ala Ser Cys Thr Val Ser Leu Gly Gly Ala  
 20 25 30

Asn Met Ala Glu Thr His Lys Ala Met Ile Leu Gln Leu Asn Pro Ser  
 35 40 45

Glu Asn Cys Thr Trp Thr Ile Glu Arg Pro Glu Asn Lys Ser Ile Arg  
 50 55 60



Ile Ile Phe Ser Tyr Val Gln Leu Asp Pro Asp Gly Ser Cys Glu Ser  
 65 70 75 80  
 Glu Asn Ile Lys Val Phe Asp Gly Thr Ser Ser Asn Gly Pro Leu Leu  
 85 90 95  
 Gly Gln Val Cys Ser Lys Asn Asp Tyr Val Pro Val Phe Glu Ser Ser  
 100 105 110  
 Ser Ser Thr Leu Thr Phe Gln Ile Val Thr Asp Ser Ala Arg Ile Gln  
 115 120 125  
 Arg Thr Val Phe Val Phe Tyr Tyr Phe Phe Ser Pro Asn Ile Ser Ile  
 130 135 140  
 Pro Asn Cys Gly Gly Tyr Leu Asp Thr Leu Glu Gly Ser Phe Thr Ser  
 145 150 155 160  
 Pro Asn Tyr Pro Lys Pro His Pro Glu Leu Ala Tyr Cys Val Trp His  
 165 170 175  
 Ile Gln Val Glu Lys Asp Tyr Lys Ile Lys Leu Asn Phe Lys Glu Ile  
 180 185 190  
 Phe Leu Glu Ile Asp Lys Gln Cys Lys Phe Asp Phe Leu Ala Ile Tyr  
 195 200 205  
 Asp Gly Pro Ser Thr Asn Ser Gly Leu Ile Gly Gln Val Cys Gly Arg  
 210 215 220  
 Val Thr Pro Thr Phe Glu Ser Ser Ser Asn Ser Leu Thr Val Val Leu  
 225 230 235 240  
 Ser Thr Asp Tyr Ala Asn Ser Tyr Arg Gly Phe Ser Ala Ser Tyr Thr  
 245 250 255  
 Ser Ile Tyr Ala Glu Asn Ile Asn Thr Thr Ser Leu Thr Cys Ser Ser  
 260 265 270  
 Asp Arg Met Arg Val Ile Ile Ser Lys Ser Tyr Leu Glu Ala Phe Asn  
 275 280 285  
 Ser Asn Gly Asn Asn Leu Gln Leu Lys Asp Pro Thr Trp Gln Thr Lys  
 290 295 300  
 Ile Ile Lys Cys Cys Gly Ile Phe Cys Pro Ser  
 305 310 315

<210> 60  
 <211> 327  
 <212> PRT  
 <213> Homo sapiens

<400> 60  
 Met Ala Glu Leu Pro Gly Pro Phe Leu Cys Gly Ala Leu Leu Gly Phe  
 1 5 10 15  
 Leu Cys Leu Ser Gly Leu Ala Val Glu Val Lys Val Pro Thr Glu Pro

20

25

43

30

Leu Ser Thr Pro Leu Gly Lys Thr Ala Glu Leu Thr Cys Thr Tyr Ser  
 35 40 45  
 Thr Ser Val Gly Asp Ser Phe Ala Leu Glu Trp Ser Phe Val Gln Pro  
 50 55 60  
 Gly Lys Pro Ile Ser Glu Ser His Pro Ile Leu Tyr Phe Thr Asn Gly  
 65 70 75 80  
 His Leu Tyr Pro Thr Gly Ser Lys Ser Lys Arg Val Ser Leu Leu Gln  
 85 90 95  
 Asn Pro Pro Thr Val Gly Val Ala Thr Leu Lys Leu Thr Asp Val His  
 100 105 110  
 Pro Ser Asp Thr Gly Thr Tyr Leu Cys Gln Val Asn Asn Pro Pro Asp  
 115 120 125  
 Phe Tyr Thr Asn Gly Leu Gly Leu Ile Asn Leu Thr Val Leu Val Pro  
 130 135 140  
 Pro Ser Asn Pro Leu Cys Ser Gln Ser Gly Gln Thr Ser Val Gly Gly  
 145 150 155 160  
 Ser Thr Ala Leu Arg Cys Ser Ser Ser Glu Gly Ala Pro Lys Pro Val  
 165 170 175  
 Tyr Asn Trp Val Arg Leu Gly Thr Phe Pro Thr Pro Ser Pro Gly Ser  
 180 185 190  
 Met Val Gln Asp Glu Val Ser Gly Gln Leu Ile Leu Thr Asn Leu Ser  
 195 200 205  
 Leu Thr Ser Ser Gly Thr Tyr Arg Cys Val Ala Thr Asn Gln Met Gly  
 210 215 220  
 Ser Ala Ser Cys Glu Leu Thr Leu Ser Val Thr Glu Pro Pro Gln Gly  
 225 230 235 240  
 Arg Val Ala Gly Ala Leu Ile Gly Val Leu Leu Gly Val Leu Leu Leu  
 245 250 255  
 Ser Val Ala Ala Phe Cys Leu Val Arg Phe Gln Lys Glu Arg Gly Lys  
 260 265 270  
 Lys Pro Lys Glu Thr Tyr Gly Gly Ser Asp Leu Arg Glu Asp Ala Ile  
 275 280 285  
 Ala Pro Gly Ile Ser Glu His Thr Cys Met Arg Ala Asp Ser Ser Lys  
 290 295 300  
 Gly Phe Leu Glu Arg Pro Ser Ser Ala Ser Thr Val Thr Thr Thr Lys  
 305 310 315 320  
 Ser Lys Leu Pro Met Val Val  
 325

<210> 61  
<211> 92  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (92)  
<223> Xaa equals stop translation

<400> 61  
Met Pro Ala Leu Arg His Pro Ala Trp Pro Cys Ile Phe Ser Leu Leu  
1 5 10 15  
Met Gly Ile Ser Asn Gly Tyr Phe Gly Ser Val Pro Met Ile Leu Ala  
20 25 30  
Ala Gly Lys Val Ser Pro Lys Gln Arg Glu Leu Ala Gly Asn Thr Met  
35 40 45  
Thr Val Ser Tyr Met Ser Gly Leu Thr Leu Gly Ser Ala Val Ala Tyr  
50 55 60  
Cys Thr Tyr Ser Leu Thr Arg Asp Ala His Gly Ser Cys Leu His Ala  
65 70 75 80  
Ser Thr Ala Asn Gly Ser Ile Leu Ala Gly Leu Xaa  
85 90

<210> 62  
<211> 58  
<212> PRT  
<213> Homo sapiens

<400> 62  
Met Glu Gly Ile Ile Thr Phe Leu Ile Leu Pro Leu Pro Cys Ser Pro  
1 5 10 15  
Gly Cys Pro Val Leu Thr Met Gln Lys Ala Val Ser Cys Thr Leu Glu  
20 25 30  
Val Ser Val Leu Leu Ser Trp Gly Leu Gly Tyr Ser Gly Ser Cys Leu  
35 40 45  
Ser Leu Val Pro Lys Ala Tyr Gln Val Ile  
50 55

<210> 63  
<211> 511  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (135)  
<223> Xaa equals any of the naturally occurring L-amino acids

&lt;400&gt; 63

```

Met Val Lys Ile Leu Val Val Thr Val Gln Leu Ile Leu Phe Gly Leu
 1           5           10           15

Ser Asn Gln Leu Ala Val Thr Phe Arg Glu Glu Asn Thr Ile Ala Phe
          20           25           30

Arg His Leu Phe Leu Leu Gly Tyr Ser Asp Gly Ala Asp Asp Thr Phe
          35           40           45

Ala Ala Tyr Thr Arg Glu Gln Leu Tyr Gln Ala Ile Phe His Ala Val
          50           55           60

Asp Gln Tyr Leu Ala Leu Pro Asp Val Ser Leu Gly Arg Tyr Ala Tyr
          65           70           75           80

Val Arg Gly Gly Gly Asp Pro Trp Thr Asn Gly Ser Gly Leu Ala Leu
          85           90           95

Cys Gln Arg Tyr Tyr His Arg Gly His Val Asp Pro Ala Asn Asp Thr
          100          105          110

Phe Asp Ile Asp Pro Met Val Val Thr Asp Cys Ile Gln Val Asp Pro
          115          120          125

Pro Glu Arg Pro Pro Pro Xaa Pro Ser Asp Asp Leu Thr Leu Leu Glu
          130          135          140

Ser Ser Ser Ser Tyr Lys Asn Leu Thr Leu Lys Phe His Lys Leu Val
          145          150          155          160

Asn Val Thr Ile His Phe Arg Leu Lys Thr Ile Asn Leu Gln Ser Leu
          165          170          175

Ile Asn Asn Glu Ile Pro Asp Cys Tyr Thr Phe Ser Val Leu Ile Thr
          180          185          190

Phe Asp Asn Lys Ala His Ser Gly Arg Ile Pro Ile Ser Leu Glu Thr
          195          200          205

Gln Ala His Ile Gln Glu Cys Lys His Pro Ser Val Phe Gln His Gly
          210          215          220

Asp Asn Ser Phe Arg Leu Leu Phe Asp Val Val Val Ile Leu Thr Cys
          225          230          235          240

Ser Leu Ser Phe Leu Leu Cys Ala Arg Ser Leu Leu Arg Gly Phe Leu
          245          250          255

Leu Gln Asn Glu Phe Val Gly Phe Met Trp Arg Gln Arg Gly Arg Val
          260          265          270

Ile Ser Leu Trp Glu Arg Leu Glu Phe Val Asn Gly Trp Tyr Ile Leu
          275          280          285

Leu Val Thr Ser Asp Val Leu Thr Ile Ser Gly Thr Ile Met Lys Ile
          290          295          300

```

Gly Ile Glu Ala Lys Asn Leu Ala Ser Tyr Asp Val Cys Ser Ile Leu  
 305 310 315 320  
 Leu Gly Thr Ser Thr Leu Leu Val Trp Val Gly Val Ile Arg Tyr Leu  
 325 330 335  
 Thr Phe Phe His Asn Tyr Asn Ile Leu Ile Ala Thr Leu Arg Val Ala  
 340 345 350  
 Leu Pro Ser Val Met Arg Phe Cys Cys Cys Val Ala Val Ile Tyr Leu  
 355 360 365  
 Gly Tyr Cys Phe Cys Gly Trp Ile Val Leu Gly Pro Tyr His Val Lys  
 370 375 380  
 Phe Arg Ser Leu Ser Met Val Ser Glu Cys Leu Phe Ser Leu Ile Asn  
 385 390 395 400  
 Gly Asp Asp Met Phe Val Thr Phe Ala Ala Met Gln Ala Gln Gln Gly  
 405 410 415  
 Arg Ser Ser Leu Val Trp Leu Phe Ser Gln Leu Tyr Leu Tyr Ser Phe  
 420 425 430  
 Ile Ser Leu Phe Ile Tyr Met Val Leu Ser Leu Phe Ile Ala Leu Ile  
 435 440 445  
 Thr Gly Ala Tyr Asp Thr Ile Lys His Pro Gly Gly Ala Gly Ala Glu  
 450 455 460  
 Glu Ser Glu Leu Gln Ala Tyr Ile Ala Gln Cys Gln Asp Ser Pro Thr  
 465 470 475 480  
 Ser Gly Lys Phe Arg Arg Gly Ser Ala Arg Ala Cys Ser Leu Leu Cys  
 485 490 495  
 Cys Cys Gly Arg Asp Pro Ser Glu Glu His Ser Leu Leu Val Asn  
 500 505 510

<210> 64  
 <211> 91  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (91)  
 <223> Xaa equals stop translation

<400> 64  
 Met Asn Trp Ser Phe Leu Cys Met Cys Leu Ala Cys Phe Pro Leu Asp  
 1 5 10 15  
 Leu Val Leu Gly Val Arg Tyr Ala Ile Glu Asp Cys Val Phe Leu Phe  
 20 25 30  
 His Leu Ser Pro Val Arg Gly Ala Leu Ile Leu Cys Pro Lys Leu Pro  
 35 40 45

Pro Trp Pro Trp Arg Cys Phe Cys Gly Leu Val Gly Phe Pro Cys Ala  
 50 55 60

His Ala Cys Pro Leu Ser Asp Ser Gly Phe Ala Ser Pro Cys Gln Ser  
 65 70 75 80

Val Pro Arg Leu Leu Thr Ala Leu Ala Arg Xaa  
 85 90

<210> 65

<211> 114

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (114)

<223> Xaa equals stop translation

<400> 65

Met Ala Ile Trp Val Val Phe Ile Tyr Trp Leu Leu Leu Val Phe Cys  
 1 5 10 15

Glu His Ser Cys Ile Ser Phe Arg Val Asp Val Cys Ile His Phe Ser  
 20 25 30

Cys Asn Lys Phe Tyr Leu Gly Val Glu Leu Leu Asp His Met Ala Ala  
 35 40 45

Leu Leu Thr Leu Trp Gly Thr Ala Arg Leu Leu Phe Lys Val Ser Ala  
 50 55 60

Pro Cys Ser Leu Ser Ser Ala Val Tyr Asp Gly Ser Val Ser Ser Gln  
 65 70 75 80

Pro His Gln Tyr Leu Phe Ser Val Cys Arg Trp Gly Leu Leu Glu His  
 85 90 95

His His Ile His Ser Phe Thr Tyr Tyr Leu Trp Leu Leu Leu Gln Tyr  
 100 105 110

Ser Xaa

<210> 66

<211> 51

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (51)

<223> Xaa equals stop translation

<400> 66

Met Thr Phe Gly Ile Val Val Asp Leu Thr Pro Val Phe Val Leu Val

1

5

48  
10

15

Leu Phe Leu Pro Ala Phe Leu Phe Leu Ser Leu Pro Ser Trp Ser Leu  
20 25 30

Pro Arg Asp Pro Thr His Val Lys Tyr Gly Leu Glu Asp Cys Met Asn  
35 40 45

Ala Ser Xaa  
50

&lt;210&gt; 67

&lt;211&gt; 215

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 67

Met Leu Leu Gln Val Val Arg Glu Gly Lys Phe Ser Gly Phe Leu Thr  
1 5 10 15

Ser Cys Ser Leu Leu Leu Pro Arg Ala Ala Gln Ile Leu Ala Ala Glu  
20 25 30

Ala Gly Leu Pro Ser Ser Arg Ser Phe Met Gly Phe Ala Ala Pro Phe  
35 40 45

Thr Asn Lys Arg Lys Ala Tyr Ser Glu Arg Arg Ile Met Gly Tyr Ser  
50 55 60

Met Gln Glu Met Tyr Glu Val Val Ser Asn Val Gln Glu Tyr Arg Glu  
65 70 75 80

Phe Val Pro Trp Cys Lys Lys Ser Leu Val Val Ser Ser Arg Lys Gly  
85 90 95

His Leu Lys Ala Gln Leu Glu Val Gly Phe Pro Pro Val Met Glu Arg  
100 105 110

Tyr Thr Ser Ala Val Ser Met Val Lys Pro His Met Val Lys Ala Val  
115 120 125

Cys Thr Asp Gly Lys Leu Phe Asn His Leu Glu Thr Ile Trp Arg Phe  
130 135 140

Ser Pro Gly Ile Pro Ala Tyr Pro Arg Thr Cys Thr Val Asp Phe Ser  
145 150 155 160

Ile Ser Phe Glu Phe Arg Ser Leu Leu His Ser Gln Leu Ala Thr Met  
165 170 175

Phe Phe Asp Glu Val Val Lys Gln Asn Val Ala Ala Phe Glu Arg Arg  
180 185 190

Ala Ala Thr Lys Phe Gly Pro Glu Thr Ala Ile Pro Arg Glu Leu Met  
195 200 205

Phe His Glu Val His Gln Thr  
210 215

<210> 68  
 <211> 311  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (256)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<400> 68  
 Met Gly Val Met Ala Met Leu Met Leu Pro Leu Leu Leu Gly Ile  
           1                  5                  10                  15  
 Ser Gly Leu Leu Phe Ile Tyr Gln Glu Val Ser Arg Leu Trp Ser Lys  
                   20                  25                  30  
 Ser Ala Val Gln Asn Lys Val Val Val Ile Thr Asp Ala Ile Ser Gly  
                   35                  40                  45  
 Leu Gly Lys Glu Cys Ala Arg Val Phe His Thr Gly Gly Ala Arg Leu  
           50                  55                  60  
 Val Leu Cys Gly Lys Asn Trp Glu Arg Leu Glu Asn Leu Tyr Asp Ala  
           65                  70                  75                  80  
 Leu Ile Ser Val Ala Asp Pro Ser Lys Thr Phe Thr Pro Lys Leu Val  
                   85                  90                  95  
 Leu Leu Asp Leu Ser Asp Ile Ser Cys Val Pro Asp Val Ala Lys Glu  
           100                  105                  110  
 Val Leu Asp Cys Tyr Gly Cys Val Asp Ile Leu Ile Asn Asn Ala Ser  
           115                  120                  125  
 Val Lys Val Lys Gly Pro Ala His Lys Ile Ser Leu Glu Leu Asp Lys  
           130                  135                  140  
 Lys Ile Met Asp Ala Asn Tyr Phe Gly Pro Ile Thr Leu Thr Lys Ala  
           145                  150                  155                  160  
 Leu Leu Pro Asn Met Ile Ser Arg Arg Thr Gly Gln Ile Val Leu Val  
                   165                  170                  175  
 Asn Asn Ile Gln Gly Lys Phe Gly Ile Pro Phe Arg Thr Thr Tyr Ala  
                   180                  185                  190  
 Ala Ser Lys His Ala Ala Leu Gly Phe Phe Asp Cys Leu Arg Ala Glu  
           195                  200                  205  
 Val Glu Glu Tyr Asp Val Val Ile Ser Thr Val Ser Pro Thr Phe Ile  
           210                  215                  220  
 Arg Ser Tyr His Val Tyr Pro Glu Gln Gly Asn Trp Glu Ala Ser Ile  
           225                  230                  235                  240  
 Trp Lys Phe Phe Phe Arg Lys Leu Thr Tyr Gly Val His Pro Val Xaa



24

50  
250

255

Val Ala Glu Glu Val Met Arg Thr Val Arg Arg Lys Lys Gln Glu Val  
260 265 270

Phe Met Ala Asn Pro Ile Pro Lys Ala Ala Val Tyr Val Arg Thr Phe  
275 280 285

Phe Pro Glu Phe Phe Phe Ala Val Val Ala Cys Gly Val Lys Glu Lys  
290 295 300

Leu Asn Val Pro Glu Glu Gly  
305 310

&lt;210&gt; 69

&lt;211&gt; 414

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (414)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 69

Met Arg Arg Gly Cys Ala Val Leu Gly Ala Leu Gly Leu Leu Ala Gly  
1 5 10 15

Ala Gly Val Gly Ser Trp Leu Leu Val Leu Tyr Leu Cys Pro Ala Ala  
20 25 30

Ser Gln Pro Ile Ser Gly Thr Leu Gln Asp Glu Glu Ile Thr Leu Ser  
35 40 45

Cys Ser Glu Ala Ser Ala Glu Glu Ala Leu Leu Pro Ala Leu Pro Lys  
50 55 60

Thr Val Ser Phe Arg Ile Asn Ser Glu Asp Phe Leu Leu Glu Ala Gln  
65 70 75 80

Val Arg Asp Gln Pro Arg Trp Leu Leu Val Cys His Glu Gly Trp Ser  
85 90 95

Pro Ala Leu Gly Leu Gln Ile Cys Trp Ser Leu Gly His Leu Arg Leu  
100 105 110

Thr His His Lys Gly Val Asn Leu Thr Asp Ile Lys Leu Asn Ser Ser  
115 120 125

Gln Glu Phe Ala Gln Leu Ser Pro Arg Leu Gly Gly Phe Leu Glu Glu  
130 135 140

Ala Trp Gln Pro Arg Asn Asn Cys Thr Ser Gly Gln Val Val Ser Leu  
145 150 155 160

Arg Cys Ser Glu Cys Gly Ala Arg Pro Leu Ala Ser Arg Ile Val Gly  
165 170 175

Gly Gln Ser Val Ala Pro Gly Arg Trp Pro Trp Gln Ala Ser Val Ala  
 180 185 190  
 Leu Gly Phe Arg His Thr Cys Gly Gly Ser Val Leu Ala Pro Arg Trp  
 195 200 205  
 Val Val Thr Ala Ala His Cys Met His Ser Phe Arg Leu Ala Arg Leu  
 210 215 220  
 Ser Ser Trp Arg Val His Ala Gly Leu Val Ser His Ser Ala Val Arg  
 225 230 235 240  
 Pro His Gln Gly Ala Leu Val Glu Arg Ile Ile Pro His Pro Leu Tyr  
 245 250 255  
 Ser Ala Gln Asn His Asp Tyr Asp Val Ala Leu Leu Arg Leu Gln Thr  
 260 265 270  
 Ala Leu Asn Phe Ser Asp Thr Val Gly Ala Val Cys Leu Pro Ala Lys  
 275 280 285  
 Glu Gln His Phe Pro Lys Gly Ser Arg Cys Trp Val Ser Gly Trp Gly  
 290 295 300  
 His Thr His Pro Ser His Thr Tyr Ser Ser Asp Met Leu Gln Asp Thr  
 305 310 315 320  
 Val Val Pro Leu Phe Ser Thr Gln Leu Cys Asn Ser Ser Cys Val Tyr  
 325 330 335  
 Ser Gly Ala Leu Thr Pro Arg Met Leu Cys Ala Gly Tyr Leu Asp Gly  
 340 345 350  
 Arg Ala Asp Ala Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Pro  
 355 360 365  
 Asp Gly Asp Thr Trp Arg Leu Val Gly Val Val Ser Trp Gly Arg Gly  
 370 375 380  
 Cys Ala Glu Pro Asn His Pro Gly Val Tyr Ala Lys Val Ala Glu Phe  
 385 390 395 400  
 Leu Asp Trp Ile His Asp Thr Ala Gln Asp Ser Leu Leu Xaa  
 405 410

&lt;210&gt; 70

&lt;211&gt; 61

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (61)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 70

Met Val Ala Tyr Ser Val Gln Val Leu Ala Val Phe Ile Ser Cys Ala  
 1 5 10 15

Ile Leu Thr Leu Ala Met Lys Ile Ala Trp Ile Phe Gly Leu Asn Ser  
20 25 30

Val Gln Asn Ile Thr Ala Asn Leu Ser Val Asp Gly Ser Thr Ser Gly  
35 40 45

Asn Pro Ile Gln Lys Trp Lys Val Ile Trp Ser Leu Xaa  
50 55 60

<210> 71

<211> 69

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (69)

<223> Xaa equals stop translation

<400> 71

Met Ala Ala Pro Leu Val Leu Val Leu Val Val Ala Val Thr Val Arg  
1 5 10 15

Ala Ala Leu Phe Arg Ser Ser Leu Ala Glu Phe Ile Ser Glu Arg Val  
20 25 30

Glu Val Val Ser Pro Leu Ser Ser Trp Lys Arg Val Val Glu Gly Leu  
35 40 45

Ser Leu Leu Gly Leu Gly Ser Ile Ser Val Phe Trp Ser Ser Ile Ser  
50 55 60

Trp Lys Leu His Xaa  
65

<210> 72

<211> 299

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (87)

<223> Xaa equals any of the naturally occurring L-amino acids

<220>

<221> SITE

<222> (299)

<223> Xaa equals stop translation

<400> 72

Met Phe Phe Phe Phe Asp Ser Val Gln Val Val Phe Thr Ile Cys Thr  
1 5 10 15

Ala Val Leu Ala Thr Ile Ala Phe Ala Phe Leu Leu Leu Pro Met Cys  
20 25 30

Gln Tyr Leu Thr Arg Pro Cys Ser Pro Gln Asn Lys Ile Ser Phe Gly  
35 40 45

Cys Cys Gly Arg Phe Thr Ala Ala Glu Leu Leu Ser Phe Ser Leu Ser  
50 55 60

Val Met Leu Val Leu Ile Trp Val Leu Thr Gly His Trp Leu Leu Met  
65 70 75 80

Asp Ala Leu Ala Met Gly Xaa Cys Val Ala Met Ile Ala Phe Val Arg  
85 90 95

Leu Pro Ser Leu Lys Val Ser Cys Leu Leu Leu Ser Gly Leu Leu Ile  
100 105 110

Tyr Asp Val Phe Trp Val Phe Phe Ser Ala Tyr Ile Phe Asn Ser Asn  
115 120 125

Val Met Val Lys Val Ala Thr Gln Pro Ala Asp Asn Pro Leu Asp Val  
130 135 140

Leu Ser Arg Lys Leu His Leu Gly Pro Asn Val Gly Arg Asp Val Pro  
145 150 155 160

Arg Leu Ser Leu Pro Gly Lys Leu Val Phe Pro Ser Ser Thr Gly Ser  
165 170 175

His Phe Ser Met Leu Gly Ile Gly Asp Ile Val Met Pro Gly Leu Leu  
180 185 190

Leu Cys Phe Val Leu Arg Tyr Asp Asn Tyr Lys Lys Gln Ala Ser Gly  
195 200 205

Asp Ser Cys Gly Ala Pro Gly Pro Ala Asn Ile Ser Gly Arg Met Gln  
210 215 220

Lys Val Ser Tyr Phe His Cys Thr Leu Ile Gly Tyr Phe Val Gly Leu  
225 230 235 240

Leu Thr Ala Thr Val Ala Ser Arg Ile His Arg Ala Ala Gln Pro Ala  
245 250 255

Leu Leu Tyr Leu Val Pro Phe Thr Leu Leu Pro Leu Leu Thr Met Ala  
260 265 270

Tyr Leu Lys Gly Asp Leu Arg Arg Met Trp Ser Glu Pro Phe His Ser  
275 280 285

Lys Ser Ser Ser Ser Arg Phe Leu Glu Val Xaa  
290 295

&lt;210&gt; 73

&lt;211&gt; 56

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

<221> SITE  
<222> (56)  
<223> Xaa equals stop translation

<400> 73

Met Pro Gly Gly Arg Asp Gly Leu Leu Tyr Leu Tyr His Gly Tyr Ser  
1 5 10 15

Ala Leu Leu Leu Trp Pro Val Ala Phe Leu His Leu Leu Phe Leu Ile  
20 25 30

Leu Leu Gly Met Cys Phe Ala Cys Cys Ile Pro Thr Ser Ser Ala Pro  
35 40 45

Leu His Thr Pro Trp Leu Ala Xaa  
50 55

<210> 74  
<211> 288  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (288)  
<223> Xaa equals stop translation

<400> 74

Met Arg Pro Asp Pro Arg Leu Lys Trp Ala Val Leu Val Leu Val Leu  
1 5 10 15

Val Gln Met Leu Ala Cys Trp Leu Val Arg Gly Leu Ala Trp Arg Trp  
20 25 30

Leu Leu Phe Trp Ala Tyr Ala Phe Gly Gly Cys Val Asn His Ser Leu  
35 40 45

Thr Leu Ala Ile His Asp Ile Ser His Asn Ala Ala Phe Gly Thr Gly  
50 55 60

Arg Ala Ala Arg Asn Arg Trp Leu Ala Val Phe Ala Asn Leu Pro Val  
65 70 75 80

Gly Val Pro Tyr Ala Ala Ser Phe Lys Lys Tyr His Val Asp His His  
85 90 95

Arg Tyr Leu Gly Gly Asp Gly Leu Asp Val Asp Val Pro Thr Arg Leu  
100 105 110

Glu Gly Trp Phe Phe Cys Thr Pro Ala Arg Lys Leu Leu Trp Leu Val  
115 120 125

Leu Gln Pro Phe Phe Tyr Ser Leu Arg Pro Leu Cys Val His Pro Lys  
130 135 140

Ala Val Thr Arg Met Glu Val Leu Asn Thr Leu Val Gln Leu Ala Ala  
145 150 155 160

55

Asp Leu Ala Ile Phe Ala Leu Trp Gly Leu Lys Pro Val Val Tyr Leu  
165 170 175

Leu Ala Ser Ser Phe Leu Gly Leu Gly Leu His Pro Ile Ser Gly His  
180 185 190

Phe Val Ala Glu His Tyr Met Phe Leu Lys Gly His Glu Thr Tyr Ser  
195 200 205

Tyr Tyr Gly Pro Leu Asn Trp Ile Thr Phe Asn Val Gly Tyr His Val  
210 215 220

Glu His His Asp Phe Pro Ser Ile Pro Gly Tyr Asn Leu Pro Leu Val  
225 230 235 240

Arg Lys Ile Ala Pro Glu Tyr Tyr Asp His Leu Pro Gln His His Ser  
245 250 255

Trp Val Lys Val Leu Trp Asp Phe Val Phe Glu Asp Ser Leu Gly Pro  
260 265 270

Tyr Ala Arg Val Lys Arg Val Tyr Arg Leu Ala Lys Asp Gly Leu Xaa  
275 280 285

&lt;210&gt; 75

&lt;211&gt; 58

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (37)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (58)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 75

Met Asp Met Lys Trp Phe Leu Ile Val Val Leu Ile Cys Ile Pro Leu  
1 5 10 15

Met Thr Ser Asp Ile Glu His Leu Phe Met Cys Leu Leu Pro Phe His  
20 25 30

Val Ser Ser Leu Xaa Lys Cys Leu Phe Lys Ser Phe Ala His Phe Ser  
35 40 45

Val Gly Leu Tyr Phe Val Val Glu Phe Xaa  
50 55

&lt;210&gt; 76

&lt;211&gt; 59

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (59)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 76

Met Ala Leu Val Trp Leu Cys Phe Leu Asn Ser Val Glu Gly Phe Gly  
1 5 10 15

Val Ser Arg Ala Pro Pro Leu Ser Pro Pro Leu Glu Glu Asn Ala Gln  
20 25 30

Asp Ser Gly Ala Ser Phe Arg Tyr Arg Lys Thr Lys Ile Ala Leu Phe  
35 40 45

Trp Thr Gln Phe Ser Val Thr Ser Ser Leu Xaa  
50 55

&lt;210&gt; 77

&lt;211&gt; 51

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (51)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 77

Met Leu Asn Phe Leu Leu Ser Asn Ser Leu Leu Leu Thr Ile Val Ser  
1 5 10 15

Ile Val Leu Leu Phe Leu Val Leu Val Thr Cys Gly Thr Val Gln Glu  
20 25 30

Asp Glu Arg Glu Arg Glu Arg Asp His Ser Cys Asn Phe Tyr Tyr Ser  
35 40 45

Ile Leu Xaa  
50

&lt;210&gt; 78

&lt;211&gt; 197

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 78

Met Gly Val Pro Leu Gly Leu Gly Ala Ala Trp Leu Leu Ala Trp Pro  
1 5 10 15

Gly Leu Ala Leu Pro Leu Val Ala Met Ala Ala Gly Gly Arg Trp Val  
20 25 30

Arg Gln Gln Gly Pro Arg Val Arg Arg Gly Ile Ser Arg Leu Trp Leu

35

40

57

45

Arg Val Leu Leu Arg Leu Ser Pro Met Ala Phe Arg Ala Leu Gln Gly  
 50 55 60

Cys Gly Ala Val Gly Asp Arg Gly Leu Phe Ala Leu Tyr Pro Lys Thr  
 65 70 75 80

Asn Lys Asp Gly Phe Arg Ser Arg Leu Pro Val Pro Gly Pro Arg Arg  
 85 90 95

Arg Asn Pro Arg Thr Thr Gln His Pro Leu Ala Leu Leu Ala Arg Val  
 100 105 110

Trp Val Leu Cys Lys Gly Trp Asn Trp Arg Leu Ala Arg Ala Ser Gln  
 115 120 125

Gly Leu Ala Ser His Leu Pro Pro Trp Ala Ile His Thr Leu Ala Ser  
 130 135 140

Trp Gly Leu Leu Arg Gly Glu Arg Pro Thr Arg Ile Pro Arg Leu Leu  
 145 150 155 160

Pro Arg Ser Gln Arg Gln Leu Gly Pro Pro Ala Ser Arg Gln Pro Leu  
 165 170 175

Pro Gly Thr Leu Ala Gly Arg Arg Ser Arg Thr Arg Gln Ser Arg Ala  
 180 185 190

Leu Pro Pro Trp Arg  
 195

&lt;210&gt; 79

&lt;211&gt; 63

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (51)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (63)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 79

Met Trp Ser Leu Val Ser Val Ser Val Leu Val Leu Thr Cys Ala Val  
 1 5 10 15

Asp Val Ala Glu Gly Leu Gly Trp Gly Glu Val Ser Thr Gly Gly Ile  
 20 25 30

Glu Leu Pro Arg His Met Val Leu Val Val Leu Val Glu Arg Glu Ser  
 35 40 45

Gln Arg Xaa Arg Thr Cys Ser Val Lys Thr Phe Ser Ser Arg Xaa



50

55

58

60

&lt;210&gt; 80

&lt;211&gt; 103

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (70)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (103)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 80

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Met | Ile | Ser | Ile | Val | Gly | Phe | Leu | Ser | Pro | Phe | Asn | Met | Ile | Leu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Gly | Ile | Val | Val | Val | Leu | Val | Phe | Thr | Gly | Phe | Val | Trp | Ala | Ala |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| His | Asn | Lys | Asp | Val | Leu | Arg | Arg | Met | Lys | Lys | Arg | Tyr | Pro | Thr | Thr |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Phe | Val | Met | Val | Val | Met | Leu | Ala | Ser | Tyr | Phe | Leu | Ile | Ser | Met | Phe |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Gly | Val | Met | Val | Xaa | Val | Phe | Gly | Ile | Thr | Phe | Pro | Leu | Leu | Leu |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Phe | Ile | His | Ala | Ser | Leu | Arg | Leu | Arg | Asn | Leu | Lys | Asn | Lys | Leu |
|     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |

|     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|
| Glu | Asn | Lys | Met | Glu | Gly | Xaa |
|     |     |     | 100 |     |     |     |

&lt;210&gt; 81

&lt;211&gt; 123

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 81

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Ile | Leu | Gly | Gly | Ile | Val | Val | Val | Leu | Val | Phe | Thr | Gly | Phe | Val |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Trp | Ala | Ala | His | Asn | Lys | Asp | Val | Leu | Arg | Arg | Met | Lys | Lys | Arg | Tyr |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Thr | Thr | Phe | Val | Met | Val | Val | Met | Leu | Ala | Ser | Tyr | Phe | Leu | Ile |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Met | Phe | Gly | Gly | Val | Met | Val | Phe | Val | Phe | Gly | Ile | Thr | Phe | Pro |
|     | 50  |     |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |

Leu Leu Leu Met Phe Ile His Ala Ser Leu Arg Leu Arg Asn Leu Lys  
 65 70 75 80

Asn Lys Leu Glu Asn Lys Met Glu Gly Ile Gly Leu Lys Arg Thr Pro  
 85 90 95

Met Gly Ile Val Leu Asp Ala Leu Glu Gln Gln Glu Glu Gly Ile Asn  
 100 105 110

Arg Leu Thr Asp Tyr Ile Ser Lys Val Lys Glu  
 115 120

<210> 82  
 <211> 73  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (73)  
 <223> Xaa equals stop translation

<400> 82  
 Met Pro Leu Thr Leu Leu Ile Leu Ser Cys Leu Ala Asp Trp Thr Met  
 1 5 10 15

Ala Glu Ala Glu Gly Asn Ala Ser Cys Thr Val Ser Leu Gly Gly Ala  
 20 25 30

Asn Met Ala Glu Thr His Lys Ala Met Ile Leu Gln Leu Asn Pro Ser  
 35 40 45

Glu Asn Cys Thr Trp Thr Ile Glu Arg Pro Glu Asn Lys Ser Ile Arg  
 50 55 60

Ile Ile Phe Ser Tyr Val Pro Ala Xaa  
 65 70

<210> 83  
 <211> 246  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (246)  
 <223> Xaa equals stop translation

<400> 83  
 Met Ala Glu Leu Pro Gly Pro Phe Leu Cys Gly Ala Leu Leu Gly Phe  
 1 5 10 15

Leu Cys Leu Ser Gly Leu Ala Val Glu Val Lys Val Pro Thr Glu Pro  
 20 25 30

Leu Ser Thr Pro Leu Gly Lys Thr Ala Glu Leu Thr Cys Thr Tyr Ser  
 35 40 45

Thr Ser Val Gly Asp Ser Phe Ala Leu Glu Trp Ser Phe Val Gln Pro  
 50 55 60  
 Gly Lys Pro Ile Ser Glu Ser His Pro Ile Leu Tyr Phe Thr Asn Gly  
 65 70 75 80  
 His Leu Tyr Pro Thr Gly Ser Lys Ser Lys Arg Val Ser Leu Leu Gln  
 85 90 95  
 Asn Pro Pro Thr Val Gly Val Ala Thr Leu Lys Leu Thr Asp Val His  
 100 105 110  
 Pro Ser Asp Thr Gly Thr Tyr Leu Cys Gln Val Asn Asn Pro Pro Asp  
 115 120 125  
 Phe Tyr Thr Asn Gly Leu Gly Leu Ile Asn Leu Thr Val Leu Val Pro  
 130 135 140  
 Pro Ser Asn Pro Leu Cys Ser Gln Ser Gly Gln Thr Ser Val Gly Gly  
 145 150 155 160  
 Ser Thr Ala Leu Arg Cys Ser Ser Ser Glu Gly Ala Pro Lys Pro Val  
 165 170 175  
 Tyr Asn Trp Val Arg Leu Gly Thr Phe Pro Thr Pro Ser Pro Gly Ser  
 180 185 190  
 Met Val Gln Asp Glu Val Ser Gly Gln Leu Ile Leu Thr Asn Leu Ser  
 195 200 205  
 Leu Thr Ser Ser Gly Thr Tyr Arg Cys Val Ala Thr Asn Gln Met Gly  
 210 215 220  
 Ser Ala Ser Cys Glu Leu Thr Leu Ser Val Thr Glu Pro Ser Gln Gly  
 225 230 235 240  
 Arg Val Ala Glu Leu Xaa  
 245

&lt;210&gt; 84

&lt;211&gt; 167

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (167)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 84

Met Gly Val Pro Leu Gly Leu Gly Ala Ala Trp Leu Leu Ala Trp Pro  
 1 5 10 15

Gly Leu Ala Leu Pro Leu Val Ala Met Ala Ala Gly Gly Arg Trp Val  
 20 25 30

Arg Gln Gln Gly Pro Arg Val Arg Arg Gly Ile Ser Arg Leu Trp Leu

35

61

40

45

Arg Val Leu Leu Arg Leu Ser Pro Met Ala Phe Arg Ala Leu Gln Gly  
50 55 60

Cys Gly Ala Val Gly Asp Arg Gly Leu Phe Ala Leu Tyr Pro Lys Thr  
65 70 75 80

Asn Lys Asp Gly Phe Arg Ser Arg Leu Pro Val Pro Gly Pro Arg Arg  
85 90 95

Arg Asn Pro Arg Thr Thr Gln His Pro Leu Ala Leu Leu Ala Arg Val  
100 105 110

Trp Val Leu Cys Lys Gly Trp Asn Trp Arg Leu Ala Arg Ala Ser Gln  
115 120 125

Gly Leu Ala Ser His Leu Pro Pro Trp Ala Ile His Thr Leu Ala Ser  
130 135 140

Trp Gly Leu Leu Arg Gly Glu Arg Pro Pro Glu Ser Pro Gly Tyr Tyr  
145 150 155 160

His Ala Ala Ser Ala Ser Xaa  
165

&lt;210&gt; 85

&lt;211&gt; 122

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 85

Pro Pro Ala Leu Gly Pro Val Ser Pro Gly Ala Ser Gly Ser Pro Gly  
1 5 10 15

Pro Val Ala Ala Ala Pro Ser Ser Leu Val Ala Ala Ala Ala Ser Val  
20 25 30

Ala Ala Ala Ala Gly Gly Asp Leu Gly Trp Met Ala Glu Thr Ala Ala  
35 40 45

Ile Ile Thr Asp Ala Ser Phe Leu Ser Gly Leu Ser Ala Ser Leu Leu  
50 55 60

Glu Arg Arg Pro Ala Ser Pro Leu Gly Pro Ala Gly Gly Leu Pro His  
65 70 75 80

Ala Pro Gln Asp Ser Val Pro Pro Ser Asp Ser Ala Ala Ser Asp Thr  
85 90 95

Thr Pro Leu Gly Ala Ala Val Gly Gly Pro Ser Pro Ala Ser Met Ala  
100 105 110

Pro Thr Glu Ala Pro Ser Glu Val Gly Ser  
115 120

&lt;210&gt; 86

&lt;211&gt; 346

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 86

Lys Ser Val Lys Leu Val Arg Leu Gln Val Pro Val Arg Asn Ser Arg  
 1 5 10 15

Val Asp Pro Arg Val Arg Lys Gly Phe Leu Arg Asn Val Val Ser Gly  
 20 25 30

Glu His Tyr Arg Phe Val Ser Met Trp Met Ala Arg Thr Ser Tyr Leu  
 35 40 45

Ala Ala Phe Ala Ile Met Val Ile Phe Thr Leu Ser Val Ser Met Leu  
 50 55 60

Leu Arg Tyr Ser His His Gln Ile Phe Val Phe Ile Ala Pro Leu Leu  
 65 70 75 80

Thr Val Ile Leu Ala Leu Val Gly Met Glu Ala Ile Met Ser Glu Phe  
 85 90 95

Phe Asn Asp Thr Thr Thr Ala Phe Tyr Ile Ile Leu Ile Val Trp Leu  
 100 105 110

Ala Asp Gln Tyr Asp Ala Ile Cys Cys His Thr Ser Thr Ser Lys Arg  
 115 120 125

His Trp Leu Arg Phe Phe Tyr Leu Tyr His Phe Ala Phe Tyr Ala Tyr  
 130 135 140

His Tyr Arg Phe Asn Gly Gln Tyr Ser Ser Leu Ala Leu Val Thr Ser  
 145 150 155 160

Trp Leu Phe Ile Gln His Ser Met Ile Tyr Phe Phe His His Tyr Glu  
 165 170 175

Leu Pro Ala Ile Leu Gln Gln Val Arg Ile Gln Glu Met Leu Leu Gln  
 180 185 190

Ala Pro Pro Leu Gly Pro Gly Thr Pro Thr Ala Leu Pro Asp Asp Met  
 195 200 205

Asn Asn Asn Ser Gly Ala Pro Ala Thr Ala Pro Asp Ser Ala Gly Gln  
 210 215 220

Pro Pro Ala Leu Gly Pro Val Ser Pro Gly Ala Ser Gly Ser Pro Gly  
 225 230 235 240

Pro Val Ala Ala Ala Pro Ser Ser Leu Val Ala Ala Ala Ala Ser Val  
 245 250 255

Ala Ala Ala Ala Gly Gly Asp Leu Gly Trp Met Ala Glu Thr Ala Ala  
 260 265 270

Ile Ile Thr Asp Ala Ser Phe Leu Ser Gly Leu Ser Ala Ser Leu Leu  
 275 280 285

Glu Arg Arg Pro Ala Ser Pro Leu Gly Pro Ala Gly Gly Leu Pro His  
 290 295 300

Ala Pro Gln Asp Ser Val Pro Pro Ser Asp Ser Ala Ala Ser Asp Thr  
 305 310 315 320

Thr Pro Leu Gly Ala Ala Val Gly Gly Pro Ser Pro Ala Ser Met Ala  
 325 330 335

Pro Thr Glu Ala Pro Ser Glu Val Gly Ser  
 340 345

<210> 87

<211> 259

<212> PRT

<213> Homo sapiens

<400> 87

Met Gly Pro His Ser Ile Leu Arg Thr Val His Cys Arg Pro Thr Lys  
 1 5 10 15

Thr Pro Pro Glu Pro Ser Ala Glu Pro His Pro Leu Ser Leu Leu Thr  
 20 25 30

Ser Ser Asn Thr Ser Leu Ala Gly Thr Ser Leu Gly Arg Asp Leu Thr  
 35 40 45

Pro Gly Gly Gly Lys Pro Pro Ser Gly Gln Thr Pro Arg Asn Pro Glu  
 50 55 60

Ser Pro Arg His Arg Leu Gly Ser Pro Arg Gly Arg Arg Trp Leu Ala  
 65 70 75 80

Ser Pro Thr Pro Thr Gly Ser Gly Arg Ser Gly Pro Ala Ser Arg Gly  
 85 90 95

Gln Arg Arg Leu Ser Cys Ala Ala Gln Asp Pro Thr Ser Glu Gly Ala  
 100 105 110

Ser Val Gly Ala Met Glu Ala Gly Leu Gly Pro Pro Thr Ala Ala Pro  
 115 120 125

Arg Gly Val Val Ser Glu Ala Ala Glu Ser Leu Gly Gly Thr Leu Ser  
 130 135 140

Trp Gly Ala Trp Gly Arg Pro Pro Ala Gly Pro Ser Gly Leu Ala Gly  
 145 150 155 160

Arg Arg Ser Arg Arg Glu Ala Leu Arg Pro Asp Arg Lys Glu Ala Ser  
 165 170 175

Val Met Met Ala Ala Val Ser Ala Ile Gln Pro Arg Ser Pro Pro Ala  
 180 185 190

Ala Ala Ala Thr Glu Ala Ala Ala Thr Arg Glu Leu Gly Ala Ala  
 195 200 205

Ala Thr Gly Pro Gly Leu Pro Leu Ala Pro Gly Glu Thr Gly Pro Arg

210

215

64

220

Ala Gly Gly Trp Pro Ala Glu Ser Gly Ala Val Ala Gly Ala Pro Glu  
 225 230 235 240

Leu Leu Phe Met Ser Ser Gly Ser Ala Val Gly Val Pro Gly Pro Ser  
 245 250 255

Gly Gly Ala

<210> 88

<211> 169

<212> PRT

<213> Homo sapiens

<400> 88

Met Ser Ala Pro Pro His Ser Ser Pro Ser Asp Trp Phe Gly Arg Arg  
 1 5 10 15

Pro Thr Pro Ser Pro Ser Gly Thr Gly Pro Arg Pro Trp Leu Leu Pro  
 20 25 30

Leu Met Leu Ala Pro Ala Pro His Val Pro Met Pro Glu Ala Gln Ala  
 35 40 45

Leu Leu Ser Arg Gly Pro Gln Ala Trp Arg Thr Arg Gly Glu Gly Gly  
 50 55 60

Ala Met Glu Lys Ala Leu Gln Gly Ala Pro Gly Arg Ala Gly Leu Arg  
 65 70 75 80

Pro Ala Gly Thr Arg Ala Arg Gly Pro Thr Pro Ser Arg Pro Leu Leu  
 85 90 95

His Thr Ser Ala Leu Leu Arg Asp Leu His His Gly Thr Pro Leu His  
 100 105 110

Pro Gln Asp Gly Ser Leu Gln Thr Tyr Gln Asp Pro Ser Arg Thr Phe  
 115 120 125

Arg Gly Thr Pro Pro Pro Leu Leu Ala Asp Gln Leu Lys His Leu Thr  
 130 135 140

Ser Gly Tyr Lys Pro Arg Ala Arg Pro His Thr Arg Gly Arg Lys Ala  
 145 150 155 160

Ala Phe Arg Ala Asn Pro Thr Lys Pro  
 165

<210> 89

<211> 387

<212> PRT

<213> Homo sapiens

<400> 89

Met Arg Arg Ser Thr His Leu Ser Met Pro Leu Trp Pro His Leu Gly

1 5 65 10 15  
Gly Gly Asp Arg Arg Gly Gly Arg Gly Lys Gly Glu Gly Gln Glu Gly  
20 25 30  
Phe Met Gly His Leu Leu Cys Ala Arg Pro Cys Ala Gln Leu Trp Ala  
35 40 45  
Arg Gln Ser Arg Glu Val Gly Gly Ser Pro Gly Ser Gln Cys Gly Glu  
50 55 60  
Ala Gly Trp Gly Leu Cys Lys Gly Ala Phe Ser Ile Thr Leu Pro Thr  
65 70 75 80  
Leu Cys Pro Gln Leu Arg Ile Gln Leu Gly Gly Ser Met Val Ser Met  
85 90 95  
Ser Gly Cys Arg Arg Lys Cys Arg Lys Gln Val Val Gln Lys Ala Cys  
100 105 110  
Cys Pro Gly Tyr Trp Gly Ser Arg Cys His Glu Cys Pro Gly Gly Ala  
115 120 125  
Glu Thr Pro Cys Asn Gly His Gly Thr Cys Leu Asp Gly Met Asp Arg  
130 135 140  
Asn Gly Thr Cys Val Cys Gln Glu Asn Phe Arg Gly Ser Ala Cys Gln  
145 150 155 160  
Glu Cys Gln Asp Pro Asn Arg Phe Gly Pro Asp Cys Gln Ser Val Cys  
165 170 175  
Ser Cys Val His Gly Val Cys Asn His Gly Pro Arg Gly Asp Gly Ser  
180 185 190  
Cys Leu Cys Phe Ala Gly Tyr Thr Gly Pro His Cys Asp Gln Glu Leu  
195 200 205  
Pro Val Cys Gln Glu Leu Arg Cys Pro Gln Asn Thr Gln Cys Ser Ala  
210 215 220  
Glu Ala Pro Ser Cys Arg Cys Leu Pro Gly Tyr Thr Gln Gln Gly Ser  
225 230 235 240  
Glu Cys Arg Ala Pro Asn Pro Cys Trp Pro Ser Pro Cys Ser Leu Leu  
245 250 255  
Ala Gln Cys Ser Val Ser Pro Lys Gly Gln Ala Gln Cys His Cys Pro  
260 265 270  
Glu Asn Tyr His Gly Asp Gly Met Val Cys Leu Pro Lys Asp Pro Cys  
275 280 285  
Thr Asp Asn Leu Gly Gly Cys Pro Ser Asn Ser Thr Leu Cys Val Tyr  
290 295 300  
Gln Lys Pro Gly Gln Ala Phe Cys Thr Cys Arg Pro Gly Leu Val Ser  
305 310 315 320



66

Ile Asn Ser Asn Ala Ser Ala Gly Cys Phe Ala Phe Cys Ser Pro Phe  
 325 330 335

Ser Cys Asp Arg Ser Ala Thr Cys Gln Val Thr Ala Asp Gly Lys Thr  
 340 345 350

Ser Cys Val Cys Arg Glu Ala Arg Trp Gly Met Gly Val Pro Ala Thr  
 355 360 365

Asp Thr Cys Ser Thr Arg Cys Arg Arg Pro Arg Arg Gln Ala Gly Cys  
 370 375 380

Ser Cys Ser  
 385

&lt;210&gt; 90

&lt;211&gt; 432

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 90

Met Asp Val Asp Thr Leu Leu Gly Glu Asp Val Gln Leu His Thr Val  
 1 5 10 15

Gly Gly Thr Arg Ala Gly Val Gln Gly Leu Ala Val His Thr Gly Ala  
 20 25 30

Arg His Asn Leu Val Leu Leu Leu Ala Ala Val Leu Gly Gln Asp Gly  
 35 40 45

Gln Asp Gly Arg Gly Gln Gln Asp Ala Val Gln His Val Asp His Thr  
 50 55 60

Ile Gly Gly His His Val Tyr Pro Leu Gln Arg Asp Pro Leu Gly Ser  
 65 70 75 80

Gln Gln Asp Ala Pro Leu Leu Arg Asn Val His Ser Gln Asp Leu Val  
 85 90 95

Arg His Gly Glu Asp Pro Thr Leu Gly Asp Glu Leu Leu Asn Gly Gln  
 100 105 110

Leu Ala Val Val Val Asp Val Val Pro His Gln Leu Leu Gln Phe Arg  
 115 120 125

Glu Thr Arg Cys Glu Glu Val Asp Gln Ala Ala Val Thr Gln Ala Val  
 130 135 140

His Ser Leu Ile Ala Trp Gly Lys Asp Cys Glu Gly Pro Arg Ser Val  
 145 150 155 160

Gln Asp Gly Gly Gln Pro Thr Val Leu Gln Asp Gly Phe Lys Ala Ala  
 165 170 175

Glu Gly Leu Gly Arg Gly Glu Asp Leu Ser Asp Ser Ser Leu Gly Ile  
 180 185 190

Pro Arg Gly Pro Arg Gly Gly Leu Pro Pro Gln Ala Ser Asp His Val

195

67

200

205

Glu Asp Ala Ile Ser Cys Tyr Val Val Gly Leu Val Asp Val Glu Arg  
 210 215 220  
 Leu Leu Gly Leu Val Phe Val Leu Val Gly Val Phe Arg Glu Leu Val  
 225 230 235 240  
 Lys Gly Asp Gly Asp Leu Leu Pro Gly Gln Arg Pro Pro Pro Ser Cys  
 245 250 255  
 Leu Leu Gly Pro Cys Val Leu Gln Asp Val Leu Pro Cys Asp Asp Val  
 260 265 270  
 Leu Ser Thr Glu Leu Leu Gly Lys Gly Cys Ile His Gly Pro Gly Gly  
 275 280 285  
 Glu Gly Gly Asp Gly Trp His Gln His Gly Glu Arg Ala Arg Cys Gly  
 290 295 300  
 Lys Asp Phe Pro Ala Ala Leu Val His His Gly His Gly Asp Pro Gln  
 305 310 315 320  
 Leu Gln Glu His Pro Ala Cys Leu Arg Gly Leu Leu His Leu Val Glu  
 325 330 335  
 Gln Val Ser Val Ala Gly Thr Pro Ile Pro His Leu Ala Ser Leu His  
 340 345 350  
 Thr Gln Leu Val Phe Pro Ser Ala Val Thr Trp Gln Val Ala Asp Arg  
 355 360 365  
 Ser Gln Glu Lys Gly Glu Gln Lys Ala Lys Gln Pro Ala Glu Ala Leu  
 370 375 380  
 Leu Leu Met Leu Thr Arg Pro Gly Arg Gln Val Gln Lys Ala Trp Pro  
 385 390 395 400  
 Gly Phe Trp Tyr Thr His Lys Val Glu Leu Leu Gly Gln Pro Pro Arg  
 405 410 415  
 Leu Ser Val His Gly Ser Leu Gly Arg His Thr Ile Pro Ser Pro Trp  
 420 425 430

&lt;210&gt; 91

&lt;211&gt; 62

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (51)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;400&gt; 91

68

Met Trp Ser Leu Val Ser Val Ser Val Leu Val Leu Thr Cys Ala Val  
 1 5 10 15

Asp Val Ala Glu Gly Leu Gly Trp Gly Glu Val Ser Thr Gly Gly Ile  
 20 25 30

Glu Leu Pro Arg His Met Val Leu Val Val Leu Val Glu Arg Glu Ser  
 35 40 45

Gln Arg Xaa Arg Thr Cys Ser Val Lys Thr Phe Ser Ser Arg  
 50 55 60

&lt;210&gt; 92

&lt;211&gt; 123

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 92

Met Ile Leu Gly Gly Ile Val Val Val Leu Val Phe Thr Gly Phe Val  
 1 5 10 15

Trp Ala Ala His Asn Lys Asp Val Leu Arg Arg Met Lys Lys Arg Tyr  
 20 25 30

Pro Thr Thr Phe Val Met Val Val Met Leu Ala Ser Tyr Phe Leu Ile  
 35 40 45

Ser Met Phe Gly Gly Val Met Val Phe Val Phe Gly Ile Thr Phe Pro  
 50 55 60

Leu Leu Leu Met Phe Ile His Ala Ser Leu Arg Leu Arg Asn Leu Lys  
 65 70 75 80

Asn Lys Leu Glu Asn Lys Met Glu Gly Ile Gly Leu Lys Arg Thr Pro  
 85 90 95

Met Gly Ile Val Leu Asp Ala Leu Glu Gln Gln Glu Glu Gly Ile Asn  
 100 105 110

Arg Leu Thr Asp Tyr Ile Ser Lys Val Lys Glu  
 115 120

&lt;210&gt; 93

&lt;211&gt; 72

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 93

Met Pro Leu Thr Leu Leu Ile Leu Ser Cys Leu Ala Asp Trp Thr Met  
 1 5 10 15

Ala Glu Ala Glu Gly Asn Ala Ser Cys Thr Val Ser Leu Gly Gly Ala  
 20 25 30

Asn Met Ala Glu Thr His Lys Ala Met Ile Leu Gln Leu Asn Pro Ser  
 35 40 45

Glu Asn Cys Thr Trp Thr Ile Glu Arg Pro Glu Asn Lys Ser Ile Arg  
 50 55 60

Ile Ile Phe Ser Tyr Val Pro Ala  
 65 70

<210> 94

<211> 254

<212> PRT

<213> Homo sapiens

<400> 94

Gln Leu Asp Pro Asp Gly Ser Cys Glu Ser Glu Asn Ile Lys Val Phe  
 1 5 10 15

Asp Gly Thr Ser Ser Asn Gly Pro Leu Leu Gly Gln Val Cys Ser Lys  
 20 25 30

Asn Asp Tyr Val Pro Val Phe Glu Ser Ser Ser Ser Thr Leu Thr Phe  
 35 40 45

Gln Ile Val Thr Asp Ser Ala Arg Ile Gln Arg Thr Val Phe Val Phe  
 50 55 60

Tyr Tyr Phe Phe Ser Pro Asn Ile Ser Ile Pro Asn Cys Gly Gly Tyr  
 65 70 75 80

Leu Asp Thr Leu Glu Gly Ser Phe Thr Ser Pro Asn Tyr Pro Lys Pro  
 85 90 95

His Pro Glu Leu Ala Tyr Cys Val Trp His Ile Gln Val Glu Lys Asp  
 100 105 110

Tyr Lys Ile Lys Leu Asn Phe Lys Glu Ile Phe Leu Glu Ile Asp Lys  
 115 120 125

Gln Cys Lys Phe Asp Phe Leu Ala Ile Tyr Asp Gly Pro Ser Thr Asn  
 130 135 140

Ser Gly Leu Ile Gly Gln Val Cys Gly Arg Val Thr Pro Thr Phe Glu  
 145 150 155 160

Ser Ser Ser Asn Ser Leu Thr Val Val Leu Ser Thr Asp Tyr Ala Asn  
 165 170 175

Ser Tyr Arg Gly Phe Ser Ala Ser Tyr Thr Ser Ile Tyr Ala Glu Asn  
 180 185 190

Ile Asn Thr Thr Ser Leu Thr Cys Ser Ser Asp Arg Met Arg Val Ile  
 195 200 205

Ile Ser Lys Ser Tyr Leu Glu Ala Phe Asn Ser Asn Gly Asn Asn Leu  
 210 215 220

Gln Leu Lys Asp Pro Thr Trp Gln Thr Lys Ile Ile Lys Trp Trp Gly  
 225 230 235 240

Asn Phe Leu Val Leu Leu Met Asp Val Val His Ser Glu Arg

<210> 95  
 <211> 51  
 <212> PRT  
 <213> Homo sapiens

<400> 95  
 Glu Ala Glu Gly Asn Ala Ser Cys Thr Val Ser Leu Gly Gly Ala Asn  
 1 5 10 15  
 Met Ala Glu Thr His Lys Ala Met Ile Leu Gln Leu Asn Pro Ser Glu  
 20 25 30  
 Asn Cys Thr Trp Thr Ile Glu Arg Pro Glu Asn Lys Ser Ile Arg Ile  
 35 40 45  
 Ile Phe Ser  
 50

<210> 96  
 <211> 324  
 <212> PRT  
 <213> Homo sapiens

<400> 96  
 Met Pro Leu Thr Leu Leu Ile Leu Ser Cys Leu Ala Asp Trp Thr Met  
 1 5 10 15  
 Ala Glu Ala Glu Gly Asn Ala Ser Cys Thr Val Ser Leu Gly Gly Ala  
 20 25 30  
 Asn Met Ala Glu Thr His Lys Ala Met Ile Leu Gln Leu Asn Pro Ser  
 35 40 45  
 Glu Asn Cys Thr Trp Thr Ile Glu Arg Pro Glu Asn Lys Ser Ile Arg  
 50 55 60  
 Ile Ile Phe Ser Tyr Val Gln Leu Asp Pro Asp Gly Ser Cys Glu Ser  
 65 70 75 80  
 Glu Asn Ile Lys Val Phe Asp Gly Thr Ser Ser Asn Gly Pro Leu Leu  
 85 90 95  
 Gly Gln Val Cys Ser Lys Asn Asp Tyr Val Pro Val Phe Glu Ser Ser  
 100 105 110  
 Ser Ser Thr Leu Thr Phe Gln Ile Val Thr Asp Ser Ala Arg Ile Gln  
 115 120 125  
 Arg Thr Val Phe Val Phe Tyr Tyr Phe Phe Ser Pro Asn Ile Ser Ile  
 130 135 140  
 Pro Asn Cys Gly Gly Tyr Leu Asp Thr Leu Glu Gly Ser Phe Thr Ser  
 145 150 155 160  
 Pro Asn Tyr Pro Lys Pro His Pro Glu Leu Ala Tyr Cys Val Trp His

165

71  
170

175

Ile Gln Val Glu Lys Asp Tyr Lys Ile Lys Leu Asn Phe Lys Glu Ile  
180 185 190

Phe Leu Glu Ile Asp Lys Gln Cys Lys Phe Asp Phe Leu Ala Ile Tyr  
195 200 205

Asp Gly Pro Ser Thr Asn Ser Gly Leu Ile Gly Gln Val Cys Gly Arg  
210 215 220

Val Thr Pro Thr Phe Glu Ser Ser Ser Asn Ser Leu Thr Val Val Leu  
225 230 235 240

Ser Thr Asp Tyr Ala Asn Ser Tyr Arg Gly Phe Ser Ala Ser Tyr Thr  
245 250 255

Ser Ile Tyr Ala Glu Asn Ile Asn Thr Thr Ser Leu Thr Cys Ser Ser  
260 265 270

Asp Arg Met Arg Val Ile Ile Ser Lys Ser Tyr Leu Glu Ala Phe Asn  
275 280 285

Ser Asn Gly Asn Asn Leu Gln Leu Lys Asp Pro Thr Trp Gln Thr Lys  
290 295 300

Ile Ile Lys Trp Trp Gly Asn Phe Leu Val Leu Leu Met Asp Val Val  
305 310 315 320

His Ser Glu Arg

<210> 97

<211> 245

<212> PRT

<213> Homo sapiens

<400> 97

Met Ala Glu Leu Pro Gly Pro Phe Leu Cys Gly Ala Leu Leu Gly Phe  
1 5 10 15

Leu Cys Leu Ser Gly Leu Ala Val Glu Val Lys Val Pro Thr Glu Pro  
20 25 30

Leu Ser Thr Pro Leu Gly Lys Thr Ala Glu Leu Thr Cys Thr Tyr Ser  
35 40 45

Thr Ser Val Gly Asp Ser Phe Ala Leu Glu Trp Ser Phe Val Gln Pro  
50 55 60

Gly Lys Pro Ile Ser Glu Ser His Pro Ile Leu Tyr Phe Thr Asn Gly  
65 70 75 80

His Leu Tyr Pro Thr Gly Ser Lys Ser Lys Arg Val Ser Leu Leu Gln  
85 90 95

Asn Pro Pro Thr Val Gly Val Ala Thr Leu Lys Leu Thr Asp Val His  
100 105 110

Pro Ser Asp Thr Gly Thr Tyr Leu Cys Gln Val Asn Asn Pro Pro Asp  
 115 120 125

Phe Tyr Thr Asn Gly Leu Gly Leu Ile Asn Leu Thr Val Leu Val Pro  
 130 135 140

Pro Ser Asn Pro Leu Cys Ser Gln Ser Gly Gln Thr Ser Val Gly Gly  
 145 150 155 160

Ser Thr Ala Leu Arg Cys Ser Ser Ser Glu Gly Ala Pro Lys Pro Val  
 165 170 175

Tyr Asn Trp Val Arg Leu Gly Thr Phe Pro Thr Pro Ser Pro Gly Ser  
 180 185 190

Met Val Gln Asp Glu Val Ser Gly Gln Leu Ile Leu Thr Asn Leu Ser  
 195 200 205

Leu Thr Ser Ser Gly Thr Tyr Arg Cys Val Ala Thr Asn Gln Met Gly  
 210 215 220

Ser Ala Ser Cys Glu Leu Thr Leu Ser Val Thr Glu Pro Ser Gln Gly  
 225 230 235 240

Arg Val Ala Glu Leu  
 245

<210> 98  
 <211> 10  
 <212> PRT  
 <213> Homo sapiens

<400> 98  
 Leu Phe Leu Leu Gly Tyr Ser Asp Gly Ala  
 1 5 10

<210> 99  
 <211> 132  
 <212> PRT  
 <213> Homo sapiens

<400> 99  
 Leu Asn Asn Ser Pro Leu Tyr Glu Asn Thr Thr Phe Tyr Leu Ser Thr  
 1 5 10 15

His Gln Val Met Ala Ile Trp Val Val Phe Ile Tyr Trp Leu Leu Leu  
 20 25 30

Val Phe Cys Glu His Ser Cys Ile Ser Phe Arg Val Asp Val Cys Ile  
 35 40 45

His Phe Ser Cys Asn Lys Phe Tyr Leu Gly Val Glu Leu Leu Asp His  
 50 55 60

Met Ala Ala Leu Leu Thr Leu Trp Gly Thr Ala Arg Leu Leu Phe Lys  
 65 70 75 80

Val Ser Ala Pro Cys Ser Leu Ser Ser Ala Val Tyr Asp Gly Ser Val  
85 90 95

Ser Ser Gln Pro His Gln Tyr Leu Phe Ser Val Cys Arg Trp Gly Leu  
100 105 110

Leu Glu His His His Ile His Ser Phe Thr Tyr Tyr Leu Trp Leu Leu  
115 120 125

Leu Gln Tyr Ser  
130

<210> 100

<211> 38

<212> PRT

<213> Homo sapiens

<400> 100

Leu Leu Asn Lys Thr Thr Phe Tyr Leu Pro Met Ala Arg Gln Val Phe  
1 5 10 15

Phe Gln Leu Ser Pro Ile His Pro Val Pro Ser Asn Leu Ser Met Gly  
20 25 30

Trp Asn Leu Thr Leu Gly  
35

<210> 101

<211> 88

<212> PRT

<213> Homo sapiens

<400> 101

Leu Leu Asn Lys Thr Thr Phe Tyr Leu Pro Met Ala Arg Gln Val Phe  
1 5 10 15

Phe Gln Leu Ser Pro Ile His Pro Val Pro Ser Asn Leu Ser Met Gly  
20 25 30

Trp Asn Leu Thr Leu Gly Met Thr Phe Gly Ile Val Val Asp Leu Thr  
35 40 45

Pro Val Phe Val Leu Val Leu Phe Leu Pro Ala Phe Leu Phe Leu Ser  
50 55 60

Leu Pro Ser Trp Ser Leu Pro Arg Asp Pro Thr His Val Lys Tyr Gly  
65 70 75 80

Leu Glu Asp Cys Met Asn Ala Ser  
85

<210> 102

<211> 47

<212> PRT

<213> Homo sapiens



&lt;400&gt; 102

Asn Ser Ala Arg Ala Ala Ala Glu Gly Arg Gly Ser Leu Arg Thr Pro  
1 5 10 15

Gly Phe Arg Gly Gly Gly Val Leu Tyr Trp Asp Ala Gly Ala Ala Gly  
20 25 30

Thr Gly Ser Asn His Ala Leu Gly Ala Asn Val Glu Leu Trp Ile  
35 40 45

&lt;210&gt; 103

&lt;211&gt; 262

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 103

Asn Ser Ala Arg Ala Ala Ala Glu Gly Arg Gly Ser Leu Arg Thr Pro  
1 5 10 15

Gly Phe Arg Gly Gly Gly Val Leu Tyr Trp Asp Ala Gly Ala Ala Gly  
20 25 30

Thr Gly Ser Asn His Ala Leu Gly Ala Asn Val Glu Leu Trp Ile Met  
35 40 45

Leu Leu Gln Val Val Arg Glu Gly Lys Phe Ser Gly Phe Leu Thr Ser  
50 55 60

Cys Ser Leu Leu Leu Pro Arg Ala Ala Gln Ile Leu Ala Ala Glu Ala  
65 70 75 80

Gly Leu Pro Ser Ser Arg Ser Phe Met Gly Phe Ala Ala Pro Phe Thr  
85 90 95

Asn Lys Arg Lys Ala Tyr Ser Glu Arg Arg Ile Met Gly Tyr Ser Met  
100 105 110

Gln Glu Met Tyr Glu Val Val Ser Asn Val Gln Glu Tyr Arg Glu Phe  
115 120 125

Val Pro Trp Cys Lys Lys Ser Leu Val Val Ser Ser Arg Lys Gly His  
130 135 140

Leu Lys Ala Gln Leu Glu Val Gly Phe Pro Pro Val Met Glu Arg Tyr  
145 150 155 160

Thr Ser Ala Val Ser Met Val Lys Pro His Met Val Lys Ala Val Cys  
165 170 175

Thr Asp Gly Lys Leu Phe Asn His Leu Glu Thr Ile Trp Arg Phe Ser  
180 185 190

Pro Gly Ile Pro Ala Tyr Pro Arg Thr Cys Thr Val Asp Phe Ser Ile  
195 200 205

Ser Phe Glu Phe Arg Ser Leu Leu His Ser Gln Leu Ala Thr Met Phe  
210 215 220

Phe Asp Glu Val Val Lys Gln Asn Val Ala Ala Phe Glu Arg Arg Ala  
225 230 235 240

Ala Thr Lys Phe Gly Pro Glu Thr Ala Ile Pro Arg Glu Leu Met Phe  
245 250 255

His Glu Val His Gln Thr  
260

<210> 104

<211> 34

<212> PRT

<213> Homo sapiens

<400> 104

Arg Trp Ile Phe Phe Gln Lys Cys Arg Pro Ile Leu Ile Lys Phe Val  
1 5 10 15

Ile Asn His Trp Gly Gly Gln Ala Pro Trp Ile Arg Ser Ala Phe Gly  
20 25 30

Asp Thr

<210> 105

<211> 345

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (290)

<223> Xaa equals any of the naturally occurring L-amino acids

<400> 105

Arg Trp Ile Phe Phe Gln Lys Cys Arg Pro Ile Leu Ile Lys Phe Val  
1 5 10 15

Ile Asn His Trp Gly Gly Gln Ala Pro Trp Ile Arg Ser Ala Phe Gly  
20 25 30

Asp Thr Met Gly Val Met Ala Met Leu Met Leu Pro Leu Leu Leu  
35 40 45

Gly Ile Ser Gly Leu Leu Phe Ile Tyr Gln Glu Val Ser Arg Leu Trp  
50 55 60

Ser Lys Ser Ala Val Gln Asn Lys Val Val Val Ile Thr Asp Ala Ile  
65 70 75 80

Ser Gly Leu Gly Lys Glu Cys Ala Arg Val Phe His Thr Gly Gly Ala  
85 90 95

Arg Leu Val Leu Cys Gly Lys Asn Trp Glu Arg Leu Glu Asn Leu Tyr  
100 105 110

Asp Ala Leu Ile Ser Val Ala Asp Pro Ser Lys Thr Phe Thr Pro Lys  
 115 120 125  
 Leu Val Leu Leu Asp Leu Ser Asp Ile Ser Cys Val Pro Asp Val Ala  
 130 135 140  
 Lys Glu Val Leu Asp Cys Tyr Gly Cys Val Asp Ile Leu Ile Asn Asn  
 145 150 155 160  
 Ala Ser Val Lys Val Lys Gly Pro Ala His Lys Ile Ser Leu Glu Leu  
 165 170 175  
 Asp Lys Lys Ile Met Asp Ala Asn Tyr Phe Gly Pro Ile Thr Leu Thr  
 180 185 190  
 Lys Ala Leu Leu Pro Asn Met Ile Ser Arg Arg Thr Gly Gln Ile Val  
 195 200 205  
 Leu Val Asn Asn Ile Gln Gly Lys Phe Gly Ile Pro Phe Arg Thr Thr  
 210 215 220  
 Tyr Ala Ala Ser Lys His Ala Ala Leu Gly Phe Phe Asp Cys Leu Arg  
 225 230 235 240  
 Ala Glu Val Glu Glu Tyr Asp Val Val Ile Ser Thr Val Ser Pro Thr  
 245 250 255  
 Phe Ile Arg Ser Tyr His Val Tyr Pro Glu Gln Gly Asn Trp Glu Ala  
 260 265 270  
 Ser Ile Trp Lys Phe Phe Phe Arg Lys Leu Thr Tyr Gly Val His Pro  
 275 280 285  
 Val Xaa Val Ala Glu Glu Val Met Arg Thr Val Arg Arg Lys Lys Gln  
 290 295 300  
 Glu Val Phe Met Ala Asn Pro Ile Pro Lys Ala Ala Val Tyr Val Arg  
 305 310 315 320  
 Thr Phe Phe Pro Glu Phe Phe Phe Ala Val Val Ala Cys Gly Val Lys  
 325 330 335  
 Glu Lys Leu Asn Val Pro Glu Glu Gly  
 340 345

&lt;210&gt; 106

&lt;211&gt; 29

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 106

Asn Ile Gln Gly Lys Phe Gly Ile Pro Phe Arg Thr Thr Tyr Ala Ala  
 1 5 10 15

Ser Lys His Ala Ala Leu Gly Phe Phe Asp Cys Leu Arg  
 20 25

<210> 107  
 <211> 480  
 <212> PRT  
 <213> Homo sapiens

<400> 107

```

Asp Pro Arg Val Arg Ala Cys Leu Ser Thr Gln Arg Asp Ile Ser Ser
 1              5              10              15

Arg Ala Ile Thr Gln Pro Gln Arg Arg Asn Pro Asn Leu Thr Phe Cys
      20              25              30

Cys Cys Phe Ser Thr Ile Leu Trp Val Leu Asp Trp Leu Ser Gln Ala
      35              40              45

Cys Cys Pro Ala Ala Ser Leu Pro Val Ser Phe Ser Gln Ala Val Cys
      50              55              60

Trp Arg Ser Met Arg Arg Gly Cys Ala Val Leu Gly Ala Leu Gly Leu
      65              70              75              80

Leu Ala Gly Ala Gly Val Gly Ser Trp Leu Leu Val Leu Tyr Leu Cys
      85              90              95

Pro Ala Ala Ser Gln Pro Ile Ser Gly Thr Leu Gln Asp Glu Glu Ile
      100             105             110

Thr Leu Ser Cys Ser Glu Ala Ser Ala Glu Glu Ala Leu Leu Pro Ala
      115             120             125

Leu Pro Lys Thr Val Ser Phe Arg Ile Asn Ser Glu Asp Phe Leu Leu
      130             135             140

Glu Ala Gln Val Arg Asp Gln Pro Arg Trp Leu Leu Val Cys His Glu
      145             150             155             160

Gly Trp Ser Pro Ala Leu Gly Leu Gln Ile Cys Trp Ser Leu Gly His
      165             170             175

Leu Arg Leu Thr His His Lys Gly Val Asn Leu Thr Asp Ile Lys Leu
      180             185             190

Asn Ser Ser Gln Glu Phe Ala Gln Leu Ser Pro Arg Leu Gly Gly Phe
      195             200             205

Leu Glu Glu Ala Trp Gln Pro Arg Asn Asn Cys Thr Ser Gly Gln Val
      210             215             220

Val Ser Leu Arg Cys Ser Glu Cys Gly Ala Arg Pro Leu Ala Ser Arg
      225             230             235             240

Ile Val Gly Gly Gln Ser Val Ala Pro Gly Arg Trp Pro Trp Gln Ala
      245             250             255

Ser Val Ala Leu Gly Phe Arg His Thr Cys Gly Gly Ser Val Leu Ala
      260             265             270

Pro Arg Trp Val Val Thr Ala Ala His Cys Met His Ser Phe Arg Leu
      275             280             285
  
```

Ala Arg Leu Ser Ser Trp Arg Val His Ala Gly Leu Val Ser His Ser  
 290 295 300

Ala Val Arg Pro His Gln Gly Ala Leu Val Glu Arg Ile Ile Pro His  
 305 310 315 320

Pro Leu Tyr Ser Ala Gln Asn His Asp Tyr Asp Val Ala Leu Leu Arg  
 325 330 335

Leu Gln Thr Ala Leu Asn Phe Ser Asp Thr Val Gly Ala Val Cys Leu  
 340 345 350

Pro Ala Lys Glu Gln His Phe Pro Lys Gly Ser Arg Cys Trp Val Ser  
 355 360 365

Gly Trp Gly His Thr His Pro Ser His Thr Tyr Ser Ser Asp Met Leu  
 370 375 380

Gln Asp Thr Val Val Pro Leu Phe Ser Thr Gln Leu Cys Asn Ser Ser  
 385 390 395 400

Cys Val Tyr Ser Gly Ala Leu Thr Pro Arg Met Leu Cys Ala Gly Tyr  
 405 410 415

Leu Asp Gly Arg Ala Asp Ala Cys Gln Gly Asp Ser Gly Gly Pro Leu  
 420 425 430

Val Cys Pro Asp Gly Asp Thr Trp Arg Leu Val Gly Val Val Ser Trp  
 435 440 445

Gly Arg Gly Cys Ala Glu Pro Asn His Pro Gly Val Tyr Ala Lys Val  
 450 455 460

Ala Glu Phe Leu Asp Trp Ile His Asp Thr Ala Gln Asp Ser Leu Leu  
 465 470 475 480

&lt;210&gt; 108

&lt;211&gt; 67

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 108

Asp Pro Arg Val Arg Ala Cys Leu Ser Thr Gln Arg Asp Ile Ser Ser  
 1 5 10 15

Arg Ala Ile Thr Gln Pro Gln Arg Arg Asn Pro Asn Leu Thr Phe Cys  
 20 25 30

Cys Cys Phe Ser Thr Ile Leu Trp Val Leu Asp Trp Leu Ser Gln Ala  
 35 40 45

Cys Cys Pro Ala Ala Ser Leu Pro Val Ser Phe Ser Gln Ala Val Cys  
 50 55 60

Trp Arg Ser  
65

<210> 109  
<211> 30  
<212> PRT  
<213> Homo sapiens

<400> 109  
Thr Cys Gly Gly Ser Val Leu Ala Pro Arg Trp Val Val Thr Ala Ala  
1 5 10 15

His Cys Met His Ser Phe Arg Leu Ala Arg Leu Ser Ser Trp  
20 25 30

<210> 110  
<211> 30  
<212> PRT  
<213> Homo sapiens

<400> 110  
Cys Ala Gly Tyr Leu Asp Gly Arg Ala Asp Ala Cys Gln Gly Asp Ser  
1 5 10 15

Gly Gly Pro Leu Val Cys Pro Asp Gly Asp Thr Trp Arg Leu  
20 25 30

<210> 111  
<211> 72  
<212> PRT  
<213> Homo sapiens

<400> 111  
Cys Arg Asn Ser Ala Arg Ala Phe Ser Gly Leu Ser Met Val Ala Tyr  
1 5 10 15

Ser Val Gln Val Leu Ala Val Phe Ile Ser Cys Ala Ile Leu Thr Leu  
20 25 30

Ala Met Lys Ile Ala Trp Ile Phe Gly Leu Asn Ser Val Gln Asn Ile  
35 40 45

Thr Ala Asn Leu Ser Val Asp Gly Ser Thr Ser Gly Asn Pro Ile Gln  
50 55 60

Lys Trp Lys Val Ile Trp Ser Leu  
65 70

<210> 112  
<211> 12  
<212> PRT  
<213> Homo sapiens

<400> 112  
Cys Arg Asn Ser Ala Arg Ala Phe Ser Gly Leu Ser

1

5

80  
10

<210> 113  
<211> 351  
<212> PRT  
<213> Homo sapiens

&lt;400&gt; 113

Met Ile Thr Asp Ala Leu Thr Ala Ile Ala Leu Tyr Phe Ala Ile Gln  
1 5 10 15

Asp Phe Asn Lys Val Val Phe Lys Lys Gln Lys Leu Leu Leu Glu Leu  
20 25 30

Asp Gln Tyr Ala Pro Asp Val Ala Glu Leu Ile Arg Thr Pro Met Glu  
35 40 45

Met Arg Tyr Ile Pro Leu Lys Val Ala Leu Phe Tyr Leu Leu Asn Pro  
50 55 60

Tyr Thr Ile Leu Ser Cys Val Ala Lys Ser Thr Cys Ala Ile Asn Asn  
65 70 75 80

Thr Leu Ile Ala Phe Phe Ile Leu Thr Thr Ile Lys Gly Ser Ala Phe  
85 90 95

Leu Ser Ala Ile Phe Leu Ala Leu Ala Thr Tyr Gln Ser Leu Tyr Pro  
100 105 110

Leu Thr Leu Phe Val Pro Gly Leu Leu Tyr Leu Leu Gln Arg Gln Tyr  
115 120 125

Ile Pro Val Lys Met Lys Ser Lys Ala Phe Trp Ile Phe Ser Trp Glu  
130 135 140

Tyr Ala Met Met Tyr Val Gly Ser Leu Val Val Ile Ile Cys Leu Ser  
145 150 155 160

Phe Phe Leu Leu Ser Ser Trp Asp Phe Ile Pro Ala Val Tyr Gly Phe  
165 170 175

Ile Leu Ser Val Pro Asp Leu Thr Pro Asn Ile Gly Leu Phe Trp Tyr  
180 185 190

Phe Phe Ala Glu Met Phe Glu His Phe Ser Leu Phe Phe Val Cys Val  
195 200 205

Phe Gln Ile Asn Val Phe Phe Tyr Thr Ile Pro Leu Ala Ile Lys Leu  
210 215 220

Lys Glu His Pro Ile Phe Phe Met Phe Ile Gln Ile Ala Val Ile Ala  
225 230 235 240

Ile Phe Lys Ser Tyr Pro Thr Val Gly Asp Val Ala Leu Tyr Met Ala  
245 250 255

Phe Phe Pro Val Trp Asn His Leu Tyr Arg Phe Leu Arg Asn Ile Phe  
260 265 270

Val Leu Thr Cys Ile Ile Ile Val Cys Ser Leu Leu Phe Pro Val Leu  
275 280 285

Trp His Leu Trp Ile Tyr Pro Gly Asn Ala Asn Ser Asn Phe Phe Tyr  
290 295 300

Ala Ile Thr Leu Thr Phe Asn Val Gly Gln Ile Leu Leu Ile Ser Asp  
305 310 315 320

Tyr Phe Tyr Ala Phe Leu Arg Arg Glu Tyr Tyr Leu Thr His Gly Leu  
325 330 335

Tyr Leu Thr Ala Lys Asp Gly Thr Glu Ala Met Leu Val Leu Lys  
340 345 350

<210> 114

<211> 81

<212> PRT

<213> Homo sapiens

<400> 114

Pro Thr Arg Pro Arg Ala Pro Ala Pro Val Ile Met Ala Ala Pro Leu  
1 5 10 15

Val Leu Val Leu Val Val Ala Val Thr Val Arg Ala Ala Leu Phe Arg  
20 25 30

Ser Ser Leu Ala Glu Phe Ile Ser Glu Arg Val Glu Val Val Ser Pro  
35 40 45

Leu Ser Ser Trp Lys Arg Val Val Glu Gly Leu Ser Leu Leu Gly Leu  
50 55 60

Gly Ser Ile Ser Val Phe Trp Ser Ser Ile Ser Trp Lys Leu His Ser  
65 70 75 80

Leu

<210> 115

<211> 11

<212> PRT

<213> Homo sapiens

<400> 115

Pro Thr Arg Pro Arg Ala Pro Ala Pro Val Ile  
1 5 10

<210> 116

<211> 322

<212> PRT

<213> Homo sapiens

<400> 116

Ile Tyr Leu Phe His Phe Leu Ile Asp Tyr Ala Glu Leu Val Phe Met



1 5 82 10 15  
Ile Thr Asp Ala Leu Thr Ala Ile Ala Leu Tyr Phe Ala Ile Gln Asp  
20 25 30  
Phe Asn Lys Val Val Phe Lys Lys Gln Lys Leu Leu Leu Glu Leu Asp  
35 40 45  
Gln Tyr Ala Pro Asp Val Ala Glu Leu Ile Arg Thr Pro Met Glu Met  
50 55 60  
Arg Tyr Ile Pro Leu Lys Val Ala Leu Phe Tyr Leu Leu Asn Pro Tyr  
65 70 75 80  
Thr Ile Leu Ser Cys Val Ala Lys Ser Thr Cys Ala Ile Asn Asn Thr  
85 90 95  
Leu Ile Ala Phe Phe Ile Leu Thr Thr Ile Lys Gly Ser Ala Phe Leu  
100 105 110  
Ser Ala Ile Phe Leu Ala Leu Ala Thr Tyr Gln Ser Leu Tyr Pro Leu  
115 120 125  
Thr Leu Phe Val Pro Gly Leu Leu Tyr Leu Leu Gln Arg Gln Tyr Ile  
130 135 140  
Pro Val Lys Met Lys Ser Lys Ala Phe Trp Ile Phe Ser Trp Glu Tyr  
145 150 155 160  
Ala Met Met Tyr Val Gly Ser Leu Val Val Ile Ile Cys Leu Ser Phe  
165 170 175  
Phe Leu Leu Ser Ser Trp Asp Phe Ile Pro Ala Val Tyr Gly Phe Ile  
180 185 190  
Leu Ser Val Pro Asp Leu Thr Pro Asn Ile Gly Leu Phe Trp Tyr Phe  
195 200 205  
Phe Ala Glu Met Phe Glu His Phe Ser Leu Phe Phe Val Cys Val Phe  
210 215 220  
Gln Ile Asn Val Phe Phe Tyr Thr Ile Pro Leu Ala Ile Lys Leu Lys  
225 230 235 240  
Glu His Pro Ile Phe Phe Met Phe Ile Gln Ile Ala Val Ile Ala Ile  
245 250 255  
Phe Lys Ser Tyr Pro Thr Val Gly Asp Val Ala Leu Tyr Met Ala Phe  
260 265 270  
Phe Pro Val Trp Asn His Leu Tyr Arg Phe Leu Arg Asn Ile Phe Val  
275 280 285  
Leu Thr Cys Ile Ile Ile Val Cys Ser Leu Leu Phe Pro Val Leu Trp  
290 295 300  
His Leu Trp Ile Tyr Pro Gly Met Pro Thr Leu Ile Ser Phe Met Pro  
305 310 315 320

Ser His

<210> 117  
<211> 15  
<212> PRT  
<213> Homo sapiens

<400> 117  
Ile Tyr Leu Phe His Phe Leu Ile Asp Tyr Ala Glu Leu Val Phe  
1 5 10 15

<210> 118  
<211> 307  
<212> PRT  
<213> Homo sapiens

<400> 118  
Met Ile Thr Asp Ala Leu Thr Ala Ile Ala Leu Tyr Phe Ala Ile Gln  
1 5 10 15  
Asp Phe Asn Lys Val Val Phe Lys Lys Gln Lys Leu Leu Leu Glu Leu  
20 25 30  
Asp Gln Tyr Ala Pro Asp Val Ala Glu Leu Ile Arg Thr Pro Met Glu  
35 40 45  
Met Arg Tyr Ile Pro Leu Lys Val Ala Leu Phe Tyr Leu Leu Asn Pro  
50 55 60  
Tyr Thr Ile Leu Ser Cys Val Ala Lys Ser Thr Cys Ala Ile Asn Asn  
65 70 75 80  
Thr Leu Ile Ala Phe Phe Ile Leu Thr Thr Ile Lys Gly Ser Ala Phe  
85 90 95  
Leu Ser Ala Ile Phe Leu Ala Leu Ala Thr Tyr Gln Ser Leu Tyr Pro  
100 105 110  
Leu Thr Leu Phe Val Pro Gly Leu Leu Tyr Leu Leu Gln Arg Gln Tyr  
115 120 125  
Ile Pro Val Lys Met Lys Ser Lys Ala Phe Trp Ile Phe Ser Trp Glu  
130 135 140  
Tyr Ala Met Met Tyr Val Gly Ser Leu Val Val Ile Ile Cys Leu Ser  
145 150 155 160  
Phe Phe Leu Leu Ser Ser Trp Asp Phe Ile Pro Ala Val Tyr Gly Phe  
165 170 175  
Ile Leu Ser Val Pro Asp Leu Thr Pro Asn Ile Gly Leu Phe Trp Tyr  
180 185 190  
Phe Phe Ala Glu Met Phe Glu His Phe Ser Leu Phe Phe Val Cys Val  
195 200 205

Phe Gln Ile Asn Val Phe Phe Tyr Thr Ile Pro Leu Ala Ile Lys Leu  
 210 215 220

Lys Glu His Pro Ile Phe Phe Met Phe Ile Gln Ile Ala Val Ile Ala  
 225 230 235 240

Ile Phe Lys Ser Tyr Pro Thr Val Gly Asp Val Ala Leu Tyr Met Ala  
 245 250 255

Phe Phe Pro Val Trp Asn His Leu Tyr Arg Phe Leu Arg Asn Ile Phe  
 260 265 270

Val Leu Thr Cys Ile Ile Ile Val Cys Ser Leu Leu Phe Pro Val Leu  
 275 280 285

Trp His Leu Trp Ile Tyr Pro Gly Met Pro Thr Leu Ile Ser Phe Met  
 290 295 300

Pro Ser His  
 305

<210> 119  
 <211> 35  
 <212> PRT  
 <213> Homo sapiens

<400> 119  
 Met Ile Thr Asp Ala Leu Thr Ala Ile Ala Leu Tyr Phe Ala Ile Gln  
 1 5 10 15

Asp Phe Asn Lys Val Val Phe Lys Lys Gln Lys Leu Leu Leu Glu Leu  
 20 25 30

Asp Gln Tyr  
 35

<210> 120  
 <211> 35  
 <212> PRT  
 <213> Homo sapiens

<400> 120  
 Ala Pro Asp Val Ala Glu Leu Ile Arg Thr Pro Met Glu Met Arg Tyr  
 1 5 10 15

Ile Pro Leu Lys Val Ala Leu Phe Tyr Leu Leu Asn Pro Tyr Thr Ile  
 20 25 30

Leu Ser Cys  
 35

<210> 121  
 <211> 35  
 <212> PRT  
 <213> Homo sapiens

&lt;400&gt; 121

Val Ala Lys Ser Thr Cys Ala Ile Asn Asn Thr Leu Ile Ala Phe Phe  
1 5 10 15

Ile Leu Thr Thr Ile Lys Gly Ser Ala Phe Leu Ser Ala Ile Phe Leu  
20 25 30

Ala Leu Ala  
35

&lt;210&gt; 122

&lt;211&gt; 35

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 122

Thr Tyr Gln Ser Leu Tyr Pro Leu Thr Leu Phe Val Pro Gly Leu Leu  
1 5 10 15

Tyr Leu Leu Gln Arg Gln Tyr Ile Pro Val Lys Met Lys Ser Lys Ala  
20 25 30

Phe Trp Ile  
35

&lt;210&gt; 123

&lt;211&gt; 35

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 123

Phe Ser Trp Glu Tyr Ala Met Met Tyr Val Gly Ser Leu Val Val Ile  
1 5 10 15

Ile Cys Leu Ser Phe Phe Leu Leu Ser Ser Trp Asp Phe Ile Pro Ala  
20 25 30

Val Tyr Gly  
35

&lt;210&gt; 124

&lt;211&gt; 35

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 124

Phe Ile Leu Ser Val Pro Asp Leu Thr Pro Asn Ile Gly Leu Phe Trp  
1 5 10 15

Tyr Phe Phe Ala Glu Met Phe Glu His Phe Ser Leu Phe Phe Val Cys  
20 25 30

Val Phe Gln  
35

<210> 125  
<211> 35  
<212> PRT  
<213> Homo sapiens

<400> 125  
Ile Asn Val Phe Phe Tyr Thr Ile Pro Leu Ala Ile Lys Leu Lys Glu  
1 5 10 15  
His Pro Ile Phe Phe Met Phe Ile Gln Ile Ala Val Ile Ala Ile Phe  
20 25 30

Lys Ser Tyr  
35

<210> 126  
<211> 35  
<212> PRT  
<213> Homo sapiens

<400> 126  
Pro Thr Val Gly Asp Val Ala Leu Tyr Met Ala Phe Phe Pro Val Trp  
1 5 10 15  
Asn His Leu Tyr Arg Phe Leu Arg Asn Ile Phe Val Leu Thr Cys Ile  
20 25 30

Ile Ile Val  
35

<210> 127  
<211> 27  
<212> PRT  
<213> Homo sapiens

<400> 127  
Cys Ser Leu Leu Phe Pro Val Leu Trp His Leu Trp Ile Tyr Pro Gly  
1 5 10 15

Met Pro Thr Leu Ile Ser Phe Met Pro Ser His  
20 25

<210> 128  
<211> 391  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (180)  
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 128  
Glu Pro Thr Arg Gly Ser Ala Met Ala Glu Gln Thr Tyr Ser Trp Ala  
1 5 10 15

Tyr Ser Leu Val Asp Ser Ser Gln Val Ser Thr Phe Leu Phe Ser Ile  
 20 25 30

Leu Leu Ile Val Tyr Gly Ser Phe Arg Ser Leu Asn Met Asp Phe Glu  
 35 40 45

Asn Gln Asp Lys Glu Lys Asp Ser Asn Ser Ser Ser Gly Ser Phe Asn  
 50 55 60

Gly Asn Ser Thr Asn Asn Ser Ile Gln Thr Ile Asp Ser Thr Gln Ala  
 65 70 75 80

Leu Phe Leu Pro Ile Gly Ala Ser Val Ser Leu Leu Val Met Phe Phe  
 85 90 95

Phe Phe Asp Ser Val Gln Val Val Phe Thr Ile Cys Thr Ala Val Leu  
 100 105 110

Ala Thr Ile Ala Phe Ala Phe Leu Leu Leu Pro Met Cys Gln Tyr Leu  
 115 120 125

Thr Arg Pro Cys Ser Pro Gln Asn Lys Ile Ser Phe Gly Cys Cys Gly  
 130 135 140

Arg Phe Thr Ala Ala Glu Leu Leu Ser Phe Ser Leu Ser Val Met Leu  
 145 150 155 160

Val Leu Ile Trp Val Leu Thr Gly His Trp Leu Leu Met Asp Ala Leu  
 165 170 175

Ala Met Gly Xaa Cys Val Ala Met Ile Ala Phe Val Arg Leu Pro Ser  
 180 185 190

Leu Lys Val Ser Cys Leu Leu Leu Ser Gly Leu Leu Ile Tyr Asp Val  
 195 200 205

Phe Trp Val Phe Phe Ser Ala Tyr Ile Phe Asn Ser Asn Val Met Val  
 210 215 220

Lys Val Ala Thr Gln Pro Ala Asp Asn Pro Leu Asp Val Leu Ser Arg  
 225 230 235 240

Lys Leu His Leu Gly Pro Asn Val Gly Arg Asp Val Pro Arg Leu Ser  
 245 250 255

Leu Pro Gly Lys Leu Val Phe Pro Ser Ser Thr Gly Ser His Phe Ser  
 260 265 270

Met Leu Gly Ile Gly Asp Ile Val Met Pro Gly Leu Leu Leu Cys Phe  
 275 280 285

Val Leu Arg Tyr Asp Asn Tyr Lys Lys Gln Ala Ser Gly Asp Ser Cys  
 290 295 300

Gly Ala Pro Gly Pro Ala Asn Ile Ser Gly Arg Met Gln Lys Val Ser  
 305 310 315 320

Tyr Phe His Cys Thr Leu Ile Gly Tyr Phe Val Gly Leu Leu Thr Ala  
 325 330 335

Thr Val Ala Ser Arg Ile His Arg Ala Ala Gln Pro Ala Leu Leu Tyr  
340 345 350  
Leu Val Pro Phe Thr Leu Leu Pro Leu Leu Thr Met Ala Tyr Leu Lys  
355 360 365  
Gly Asp Leu Arg Arg Met Trp Ser Glu Pro Phe His Ser Lys Ser Ser  
370 375 380  
Ser Ser Arg Phe Leu Glu Val  
385 390

<210> 129  
<211> 93  
<212> PRT  
<213> Homo sapiens

<400> 129  
Glu Pro Thr Arg Gly Ser Ala Met Ala Glu Gln Thr Tyr Ser Trp Ala  
1 5 10 15  
Tyr Ser Leu Val Asp Ser Ser Gln Val Ser Thr Phe Leu Ile Ser Ile  
20 25 30  
Leu Leu Ile Val Tyr Gly Ser Phe Arg Ser Leu Asn Met Asp Phe Glu  
35 40 45  
Asn Gln Asp Lys Glu Lys Asp Ser Asn Ser Ser Ser Gly Ser Phe Asn  
50 55 60  
Gly Asn Ser Thr Asn Asn Ser Ile Gln Thr Ile Asp Ser Thr Gln Ala  
65 70 75 80  
Leu Phe Leu Pro Ile Gly Ala Ser Val Ser Leu Leu Val  
85 90

<210> 130  
<211> 323  
<212> PRT  
<213> Homo sapiens

<400> 130  
Met Gly Asn Ser Ala Ser Arg Asn Asp Phe Glu Trp Val Tyr Thr Asp  
1 5 10 15  
Gln Pro His Thr Gln Arg Arg Ala Arg Pro Pro Ala Lys Tyr Pro Ala  
20 25 30  
Ile Lys Ala Leu Met Arg Pro Asp Pro Arg Leu Lys Trp Ala Val Leu  
35 40 45  
Val Leu Val Leu Val Gln Met Leu Ala Cys Trp Leu Val Arg Gly Leu  
50 55 60  
Ala Trp Arg Trp Leu Leu Phe Trp Ala Tyr Ala Phe Gly Gly Cys Val  
65 70 75 80





Val Arg Ala Pro Asn Gly Ala Ser Arg Pro Thr Met Gly Asn Ser Ala  
20 25 30

Ser Arg Asn Asp Phe Glu Trp Val Tyr Thr Asp Gln Pro His Thr Gln  
35 40 45

Arg Arg Ala Arg Pro Pro Ala Lys Tyr Pro Ala Ile Lys Ala Leu Met  
50 55 60

Arg Pro Asp Pro Arg Leu Lys Trp Ala Val Leu Val Leu Val Leu Val  
65 70 75 80

Gln Met Leu Ala Cys Trp Leu Val Arg Gly Leu Ala Trp Arg Trp Leu  
85 90 95

Leu Phe Trp Ala Tyr Ala Phe Gly Gly Cys Val Asn His Ser Leu Thr  
100 105 110

Leu Ala Ile His Asp Ile Ser His Asn Ala Ala Phe Gly Thr Gly Arg  
115 120 125

Ala Ala Arg Asn Arg Trp Leu Ala Val Phe Ala Asn Leu Pro Val Gly  
130 135 140

Val Pro Tyr Ala Ala Ser Phe Lys Lys Tyr His Val Asp His His Arg  
145 150 155 160

Tyr Leu Gly Gly Asp Gly Leu Asp Val Asp Val Pro Thr Arg Leu Glu  
165 170 175

Gly Trp Phe Phe Cys Thr Pro Ala Arg Lys Leu Leu Trp Leu Val Leu  
180 185 190

Gln Pro Phe Phe Tyr Ser Leu Arg Pro Leu Cys Val His Pro Lys Ala  
195 200 205

Val Thr Arg Met Glu Val Leu Asn Thr Leu Val Gln Leu Ala Ala Asp  
210 215 220

Leu Ala Ile Phe Ala Leu Trp Gly Leu Lys Pro Val Val Tyr Leu Leu  
225 230 235 240

Ala Ser Ser Phe Leu Gly Leu Gly Leu His Pro Ile Ser Gly His Phe  
245 250 255

Val Ala Glu His Tyr Met Phe Leu Lys Gly His Glu Thr Tyr Ser Tyr  
260 265 270

Tyr Gly Pro Leu Asn Trp Ile Thr Phe Asn Val Gly Tyr His Val Glu  
275 280 285

His His Asp Phe Pro Ser Ile Pro Gly Tyr Asn Leu Pro Leu Val Arg  
290 295 300

Lys Ile Ala Pro Glu Tyr Tyr Asp His Leu Pro Gln His His Ser Trp  
305 310 315 320

Val Lys Val Leu Trp Asp Phe Val Phe Glu Asp Ser Leu Gly Pro Tyr  
325 330 335

Ala Arg Val Lys Arg Val Tyr Arg Leu Ala Lys Asp Gly Leu  
340 345 350

<210> 132  
<211> 27  
<212> PRT  
<213> Homo sapiens

<400> 132  
Leu Gln Val Pro Val Arg Asn Ser Arg Val Asp Pro Arg Val Arg Ala  
1 5 10 15

Val Arg Ala Pro Asn Gly Ala Ser Arg Pro Thr  
20 25

<210> 133  
<211> 80  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (60)  
<223> Xaa equals any of the naturally occurring L-amino acids

<400> 133  
Gly Phe Ser Phe Ser Thr Ser Leu Pro Thr Leu Val Ile Phe Trp Val  
1 5 10 15

Phe Leu Ile Ile Ala Phe Leu Met Asp Met Lys Trp Phe Leu Ile Val  
20 25 30

Val Leu Ile Cys Ile Pro Leu Met Thr Ser Asp Ile Glu His Leu Phe  
35 40 45

Met Cys Leu Leu Pro Phe His Val Ser Ser Leu Xaa Lys Cys Leu Phe  
50 55 60

Lys Ser Phe Ala His Phe Ser Val Gly Leu Tyr Phe Val Val Glu Phe  
65 70 75 80

<210> 134  
<211> 23  
<212> PRT  
<213> Homo sapiens

<400> 134  
Gly Phe Ser Phe Ser Thr Ser Leu Pro Thr Leu Val Ile Phe Trp Val  
1 5 10 15

Phe Leu Ile Ile Ala Phe Leu  
20

<210> 135  
<211> 78  
<212> PRT  
<213> Homo sapiens

<400> 135  
Arg Gln Leu Pro Glu Cys Pro Pro Ser Cys Ala Val Ser Cys Trp His  
1 5 10 15  
Trp Asp Glu Asp Met Ala Leu Val Trp Leu Cys Phe Leu Asn Ser Val  
20 25 30  
Glu Gly Phe Gly Val Ser Arg Ala Pro Pro Leu Ser Pro Pro Leu Glu  
35 40 45  
Glu Asn Ala Gln Asp Ser Gly Ala Ser Phe Arg Tyr Arg Lys Thr Lys  
50 55 60  
Ile Ala Leu Phe Trp Thr Gln Phe Ser Val Thr Ser Ser Leu  
65 70 75

<210> 136  
<211> 78  
<212> PRT  
<213> Homo sapiens

<400> 136  
Arg Gln Leu Pro Glu Cys Pro Pro Ser Cys Ala Val Ser Cys Trp His  
1 5 10 15  
Trp Asp Glu Asp Met Ala Leu Val Trp Leu Cys Phe Leu Asn Ser Val  
20 25 30  
Glu Gly Phe Gly Val Ser Arg Ala Pro Pro Leu Ser Pro Pro Leu Glu  
35 40 45  
Glu Asn Ala Gln Asp Ser Gly Ala Ser Phe Arg Tyr Arg Lys Thr Lys  
50 55 60  
Ile Ala Leu Phe Trp Thr Gln Phe Ser Val Thr Ser Ser Leu  
65 70 75

<210> 137  
<211> 20  
<212> PRT  
<213> Homo sapiens

<400> 137  
Arg Gln Leu Pro Glu Cys Pro Pro Ser Cys Ala Val Ser Cys Trp His  
1 5 10 15  
Trp Asp Glu Asp  
20

<210> 138  
 <211> 279  
 <212> PRT  
 <213> Homo sapiens

<400> 138

His Glu Val Gly Ser Ser Ser Gly Leu Leu Pro Leu Leu Leu Leu  
 1 5 10 15

Leu Leu Pro Leu Leu Ala Ala Gln Gly Gly Gly Gly Leu Gln Ala Ala  
 20 25 30

Leu Leu Ala Leu Glu Val Gly Leu Val Gly Leu Gly Ala Ser Tyr Leu  
 35 40 45

Leu Leu Cys Thr Ala Leu His Leu Pro Ser Ser Leu Phe Leu Leu Leu  
 50 55 60

Ala Gln Gly Thr Ala Leu Gly Ala Val Leu Gly Leu Ser Trp Arg Arg  
 65 70 75 80

Gly Leu Met Gly Val Pro Leu Gly Leu Gly Ala Ala Trp Leu Leu Ala  
 85 90 95

Trp Pro Gly Leu Ala Leu Pro Leu Val Ala Met Ala Ala Gly Gly Arg  
 100 105 110

Trp Val Arg Gln Gln Gly Pro Arg Val Arg Arg Gly Ile Ser Arg Leu  
 115 120 125

Trp Leu Arg Val Leu Leu Arg Leu Ser Pro Met Ala Phe Arg Ala Leu  
 130 135 140

Gln Gly Cys Gly Ala Val Gly Asp Arg Gly Leu Phe Ala Leu Tyr Pro  
 145 150 155 160

Lys Thr Asn Lys Asp Gly Phe Arg Ser Arg Leu Pro Val Pro Gly Pro  
 165 170 175

Arg Arg Arg Asn Pro Arg Thr Thr Gln His Pro Leu Ala Leu Leu Ala  
 180 185 190

Arg Val Trp Val Leu Cys Lys Gly Trp Asn Trp Arg Leu Ala Arg Ala  
 195 200 205

Ser Gln Gly Leu Ala Ser His Leu Pro Pro Trp Ala Ile His Thr Leu  
 210 215 220

Ala Ser Trp Gly Leu Leu Arg Gly Glu Arg Pro Thr Arg Ile Pro Arg  
 225 230 235 240

Leu Leu Pro Arg Ser Gln Arg Gln Leu Gly Pro Pro Ala Ser Arg Gln  
 245 250 255

Pro Leu Pro Gly Thr Leu Ala Gly Arg Arg Ser Arg Thr Arg Gln Ser  
 260 265 270

Arg Ala Leu Pro Pro Trp Arg  
 275

<210> 139  
<211> 166  
<212> PRT  
<213> Homo sapiens

<400> 139

Met Gly Val Pro Leu Gly Leu Gly Ala Ala Trp Leu Leu Ala Trp Pro  
1 5 10 15

Gly Leu Ala Leu Pro Leu Val Ala Met Ala Ala Gly Gly Arg Trp Val  
20 25 30

Arg Gln Gln Gly Pro Arg Val Arg Arg Gly Ile Ser Arg Leu Trp Leu  
35 40 45

Arg Val Leu Leu Arg Leu Ser Pro Met Ala Phe Arg Ala Leu Gln Gly  
50 55 60

Cys Gly Ala Val Gly Asp Arg Gly Leu Phe Ala Leu Tyr Pro Lys Thr  
65 70 75 80

Asn Lys Asp Gly Phe Arg Ser Arg Leu Pro Val Pro Gly Pro Arg Arg  
85 90 95

Arg Asn Pro Arg Thr Thr Gln His Pro Leu Ala Leu Leu Ala Arg Val  
100 105 110

Trp Val Leu Cys Lys Gly Trp Asn Trp Arg Leu Ala Arg Ala Ser Gln  
115 120 125

Gly Leu Ala Ser His Leu Pro Pro Trp Ala Ile His Thr Leu Ala Ser  
130 135 140

Trp Gly Leu Leu Arg Gly Glu Arg Pro Pro Glu Ser Pro Gly Tyr Tyr  
145 150 155 160

His Ala Ala Ser Ala Ser  
165

<210> 140  
<211> 225  
<212> PRT  
<213> Homo sapiens

<400> 140

Gln Gly Gly Gly Gly Leu Gln Ala Ala Leu Leu Ala Leu Glu Val Gly  
1 5 10 15

Leu Val Gly Leu Gly Ala Ser Tyr Leu Leu Leu Cys Thr Ala Leu His  
20 25 30

Leu Pro Ser Ser Leu Phe Leu Leu Leu Ala Gln Gly Thr Ala Leu Gly  
35 40 45

Ala Val Leu Gly Leu Ser Trp Arg Arg Gly Leu Met Gly Val Pro Leu  
50 55 60

Gly Leu Gly Ala Ala Trp Leu Leu Ala Trp Pro Gly Leu Ala Leu Pro  
 65 70 75 80  
 Leu Val Ala Met Ala Ala Gly Gly Arg Trp Val Arg Gln Gln Gly Pro  
 85 90 95  
 Arg Val Arg Arg Gly Ile Ser Arg Leu Trp Leu Arg Val Leu Leu Arg  
 100 105 110  
 Leu Ser Pro Met Ala Phe Arg Ala Leu Gln Gly Cys Gly Ala Val Gly  
 115 120 125  
 Asp Arg Gly Leu Phe Ala Leu Tyr Pro Lys Thr Asn Lys Asp Gly Phe  
 130 135 140  
 Arg Ser Arg Leu Pro Val Pro Gly Pro Arg Arg Arg Asn Pro Arg Thr  
 145 150 155 160  
 Thr Gln His Pro Leu Ala Leu Leu Ala Arg Val Trp Val Leu Cys Lys  
 165 170 175  
 Gly Trp Asn Trp Arg Leu Ala Arg Ala Ser Gln Gly Leu Ala Ser His  
 180 185 190  
 Leu Pro Pro Trp Ala Ile His Thr Leu Ala Ser Trp Gly Leu Leu Arg  
 195 200 205  
 Gly Glu Arg Pro Pro Glu Ser Pro Gly Tyr Tyr His Ala Ala Ser Ala  
 210 215 220  
 Ser  
 225

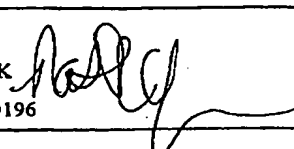
<210> 141  
 <211> 29  
 <212> PRT  
 <213> Homo sapiens

<400> 141  
 Cys Gly Gly Tyr Leu Asp Thr Leu Glu Gly Ser Phe Thr Ser Pro Asn  
 1 5 10 15

Tyr Pro Lys Pro His Pro Glu Leu Ala Tyr Cys Val Trp  
 20 25

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/22012

| <b>A. CLASSIFICATION OF SUBJECT MATTER</b><br>IPC(7) :C07H 21/04; C07K 1/00; C12P 12/06<br>US CL :530/350; 536/23.5; 435/252.3; 435/69.1<br>According to International Patent Classification (IPC) or to both national classification and IPC   |  |   |
|---|--|---|
| <b>B. FIELDS SEARCHED</b><br>Minimum documentation searched (classification system followed by classification symbols)<br>U.S. : 530/350; 536/23.5; 435/252.3; 435/69.1<br>Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched<br>Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)<br>Please See Extra Sheet.   |  |   |
| <b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>   |  |   |
| Category*   | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.   |
| X<br>---<br>Y   | US 5,663,060 A (LOLLAR et al.) 02 September 1997, columns 73-74, lines 11-12, and see attached sequence alignment.   | 11, 12<br>-----<br>17   |
| X<br>--<br>Y  | Database EST:*, Accession Number AA031520, HILLIER et al., 'Homo sapiens cDNA clone IMAGE: 470447 3', mRNA Sequence, 09 May 1997, see attached sequence alignment.   | 1-12, 14-17<br>-----<br>21  |
| X<br>--<br>Y  | Database EST:*, Accession Number AA422016, HILLIER et al., 'Homo sapiens cDNA clone IMAGE: 754761 5', mRNA sequence, 16 October 1997, see attached sequence alignment.   | 1-12, 14-17<br>-----<br>21  |
| X<br>--<br>Y  | Database EST:*, Accession Number H29528, HILLIER et al., 'Homo sapiens cDNA clone IMAGE: 52848', 17 July 1995, see attached sequence alignment   | 1-12, 14-17<br>-----<br>21  |
| <input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.  |  |   |
| * Special categories of cited documents:<br>"A" document defining the general state of the art which is not considered to be of particular relevance<br>"B" earlier document published on or after the international filing date<br>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)<br>"O" document referring to an oral disclosure, use, exhibition or other means<br>"P" document published prior to the international filing date but later than the priority date claimed | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention<br>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone<br>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art<br>"G" document member of the same patent family |   |
| Date of the actual completion of the international search<br>28 DECEMBER 1999   |  | Date of mailing of the international search report<br>09 FEB 2000   |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. (703) 305-3230   |  | Authorized officer<br>MICHAEL T. BRANNOCK <br>Telephone No. (703) 308-0196 |

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/22012

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category*    | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No.      |
|--------------|---|----------------------------|
| X<br>--<br>Y | Database EST:*, Accession number N64374, HILLIER et al.,<br>'Homo sapiens cDNA clone IMAGE: 290199 3', mRNA sequence,<br>01 March 1995, see attached sequence alignment.    | 1-12, 14-17<br>-----<br>21 |
| X<br>--<br>Y | Database EST:*, Accession Number AA350344, ADAMS et al.,<br>'Homo sapiens cDNA 5 end', mRNA sequence, 21 April 1997, see<br>attached sequence alignment.                    | 1-12, 14-17<br>-----<br>21 |
| X            | Database GenEmbl:*, Accession Number AB011109, OHARA et<br>al., 'Homo sapiens mRNA for KIAA0537 protein', complete cds.,<br>10 April 1998, see attached sequence alignment. | 1-12, 14-17 and<br>21      |

Form PCT/ISA/210 (continuation of second sheet)(July 1992)\*



# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/22012

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-12, 14-17, 21

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/22012

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

STN: MEDLINE, BIOSIS. COMERCIAL SEQ DATA BASES: EST:\*, GENBANK, GENEMBL, SWISSPROT,  
ISSUED PATENTS  
WEST1.2  
SEARCH TERMS: RUBEN SM, ROSEN CA, CHROMOSOME 12, SECRETED

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-12, 14-17, and 21 drawn to a polynucleotide of SEQ ID NO: X, vectors, host cells, polypeptides, methods of making polypeptides and methods of making host cells.

Group II, claim 13, drawn to an antibody.

Group III, claim 18, drawn to a method of diagnosis comprising the detection of a mutation in a polynucleotide.

Group IV, claim 19, drawn to a method of diagnosis comprising measuring the amount of a polypeptide.

Group V, claim 20, drawn to a method of identifying a binding partner of a polypeptide.

Group VI, claim 22, drawn to a method for identifying a biological activity.

Group VII, claim 23, drawn to a product.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

In Group I, SEQ ID NO: X corresponds to any of 31 different amino acid sequences listed in the Table beginning on page 91.

The inventions listed as Groups I-VII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Pursuant to 37 C.F.R. 1.475(d), this authority considers that the main invention in the instant application comprises the first recited product, a polynucleotide of SEQ ID NO: 11, and the first recited method of using that product, namely in the process of producing the encoded polypeptide. Further, pursuant to 37 C.F.R. 1.475(b)-(d), the ISA/US considers that the materially and functionally dissimilar products of Groups II and VII and the additional methods of Groups III and VI do not correspond to the main invention. This authority therefore considers that the several inventions do not share a special technical feature within the meaning of PCT Rule 13.2 and thus do not relate to a single general inventive concept within the meaning of PCT Rule 13.1.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: The different polypeptides of Group I are distinct molecules with different structures and features.



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

|  |   |  |
|--|---|--|
| (51) International Patent Classification <sup>7</sup> :<br><b>C07H 21/04, C07K 1/00</b>  | <b>A1</b>   | (11) International Publication Number: <b>WO 00/17222</b><br>(43) International Publication Date: 30 March 2000 (30.03.00) |
| (21) International Application Number: PCT/US99/22012<br>(22) International Filing Date: 22 September 1999 (22.09.99)<br>(30) Priority Data:<br>60/101,546      23 September 1998 (23.09.98)      US<br>60/102,895      2 October 1998 (02.10.98)      US<br>(71) Applicant (for all designated States except US): HUMAN GENOME SCIENCES, INC. [US/US]; 9410 Key West Avenue, Rockville, MD 20850 (US).<br>(72) Inventors; and<br>(75) Inventors/Applicants (for US only): RUBEN, Steven, M. [US/US]; 18528 Heritage Hills Drive, Olney, MD 20832 (US). ROSEN, Craig, A. [US/US]; 22400 Rolling Hill Road, Laytonsville, MD 20882 (US). DUAN, Roxanne, D. [US/US]; 5515 Northfield Road, Bethesda, MD 20817 (US). SHI, Yanggu [CN/US]; Apartment 102, 437 West Side Drive, Gaithersburg, MD 20878 (US). LAFLEUR, David, W. [US/US]; 3142 Quesada Street, NW, Washington, DC 20015 (US). YOUNG, Paul, E. [US/US]; 122 Beckwith Street, Gaithersburg, MD 20878 (US). NI, Jian [US/US]; 5502 Manorfield Road, Rockville, MD 20853 (US). KOMATSOULIS, George [US/US]; 9518 Garwood Street, Silver Spring, MD 20901 (US). ENDRESS, Gregory, | A. [US/US]; 9729 Claggett Farm Drive, Potomac, MD 20854 (US). SOPPET, Daniel, R. [US/US]; 15050 Stillfield Place, Centreville, VA 22020 (US).<br>(74) Agents: HOOVER, Kenley, K. et al.; Human Genome Sciences, Inc., 9410 Key West Avenue, Rockville, MD 20850 (US).<br>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).<br>Published<br><i>With a revised version of the international search report.</i><br>(88) Date of publication of the revised version of the international search report: 6 July 2000 (06.07.00) |  |
| (54) Title: 31 HUMAN SECRETED PROTEINS   |   |  |
| (57) Abstract<br><p>The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human secreted proteins.</p>  |   |  |

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

|    |                          |    |  |    |  |    |                          |
|----|--------------------------|----|--|----|--|----|--------------------------|
| AL | Albania                  | ES | Spain                                    | LS | Lesotho                                      | SI | Slovenia                 |
| AM | Armenia                  | FI | Finland                                  | LT | Lithuania                                    | SK | Slovakia                 |
| AT | Austria                  | FR | France                                   | LU | Luxembourg                                   | SN | Senegal                  |
| AU | Australia                | GA | Gabon                                    | LV | Latvia                                       | SZ | Swaziland                |
| AZ | Azerbaijan               | GB | United Kingdom                           | MC | Monaco                                       | TD | Chad                     |
| BA | Bosnia and Herzegovina   | GE | Georgia                                  | MD | Republic of Moldova                          | TG | Togo                     |
| BB | Barbados                 | GH | Ghana                                    | MG | Madagascar                                   | TJ | Tajikistan               |
| BE | Belgium                  | GN | Guinea                                   | MK | The former Yugoslav<br>Republic of Macedonia | TM | Turkmenistan             |
| BF | Burkina Faso             | GR | Greece                                   |    |  | TR | Turkey                   |
| BG | Bulgaria                 | HU | Hungary                                  | ML | Mali   | TT | Trinidad and Tobago      |
| BJ | Benin                    | IE | Ireland                                  | MN | Mongolia                                     | UA | Ukraine                  |
| BR | Brazil                   | IL | Israel                                   | MR | Mauritania                                   | UG | Uganda                   |
| BY | Belarus                  | IS | Iceland                                  | MW | Malawi                                       | US | United States of America |
| CA | Canada                   | IT | Italy                                    | MX | Mexico                                       | UZ | Uzbekistan               |
| CF | Central African Republic | JP | Japan                                    | NE | Niger  | VN | Viet Nam                 |
| CG | Congo                    | KE | Kenya                                    | NL | Netherlands                                  | YU | Yugoslavia               |
| CH | Switzerland              | KG | Kyrgyzstan                               | NO | Norway                                       | ZW | Zimbabwe                 |
| CI | Côte d'Ivoire            | KP | Democratic People's<br>Republic of Korea | NZ | New Zealand                                  |    |                          |
| CM | Cameroon                 |    |  | PL | Poland                                       |    |                          |
| CN | China                    | KR | Republic of Korea                        | PT | Portugal                                     |    |                          |
| CU | Cuba                     | KZ | Kazakstan                                | RO | Romania                                      |    |                          |
| CZ | Czech Republic           | LC | Saint Lucia                              | RU | Russian Federation                           |    |                          |
| DE | Germany                  | LI | Liechtenstein                            | SD | Sudan  |    |                          |
| DK | Denmark                  | LK | Sri Lanka                                | SE | Sweden                                       |    |                          |
| EE | Estonia                  | LR | Liberia                                  | SG | Singapore                                    |    |                          |

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/22012

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07H 21/04; C07K 1/00

US CL : 530/350; 536/23.5; 435/252.3; 435/69.1

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350; 536/23.5; 435/252.3; 435/69.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category*     | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.      |
|---------------|--|----------------------------|
| X<br>---<br>Y | US 5,663,060 A (LOLLAR et al.) 02 September 1997, columns 73-74, lines 11-12, and see attached sequence alignment.   | 11, 12<br>-----<br>17      |
| X<br>--<br>Y  | Database EST:*, Accession Number AA031520, HILLIER et al., 'Homo sapiens cDNA clone IMAGE: 470447 3', mRNA Sequence, 09 May 1997, see attached sequence alignment.     | 1-12, 14-17<br>-----<br>21 |
| X<br>--<br>Y  | Database EST:*, Accession Number AA422016, HILLIER et al., 'Homo sapiens cDNA clone IMAGE: 754761 5', mRNA sequence, 16 October 1997, see attached sequence alignment. | 1-12, 14-17<br>-----<br>21 |
| X<br>--<br>Y  | Database EST:*, Accession Number H29528, HILLIER et al., 'Homo sapiens cDNA clone IMAGE: 52848', 17 July 1995, see attached sequence alignment                         | 1-12, 14-17<br>-----<br>21 |

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

|   |  |
|---|--|
| * Special categories of cited documents:  | *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention  |
| *A* document defining the general state of the art which is not considered to be of particular relevance  | *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone   |
| *E* earlier document published on or after the international filing date  | *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | *Z* document member of the same patent family  |
| *O* document referring to an oral disclosure, use, exhibition or other means  |  |
| *P* document published prior to the international filing date but later than the priority date claimed  |  |

Date of the actual completion of the international search

28 DECEMBER 1999

Date of mailing of the international search report

04 APR 2000

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

MICHAEL T. BRANNOCK

Telephone No. (703) 308-0196

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/22012

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category*    | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim          |
|--------------|--|----------------------------|
| X<br>--<br>Y | Database EST:*, Accession number N64374, HILLIER et al.,<br>'Homo sapiens cDNA clone IMAGE: 290199 3', mRNA sequence,<br>01 March 1995, see attached sequence alignment.     | 1-12, 14-17<br>-----<br>21 |
| X<br>--<br>Y | Database EST:*, Accession Number AA350344, ADAMS et al.,<br>'Homo sapiens cDNA 5 end', mRNA sequence, 21 April 1997, see<br>attached sequence alignment.                     | 1-12, 14-17<br>-----<br>21 |
| X            | Database GenE'nb'l:*, Accession Number AB011109, OHARA et<br>al., 'Homo sapiens mRNA for KIAA0537 protein', complete cds.,<br>10 April 1998, see attached sequence alignmen. | 1-12, 14-17 and<br>21      |

# INTERNATIONAL SEARCH REPORT

International application No.

T/US99/22012

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-12, 14-17, 21

Remark on Protest

☐  
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.  
CT/US99/22012

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

STN: MEDLINE, BIOSIS. COMERCIAL SEQ DATA BASES: EST:\*, GENBANK, GENEMBL, SWISSPROT, ISSUED PATENTS

WEST1.2

SEARCH TERMS: RUBEN SM, ROSEN CA, CHROMOSOME 12, SECRETED

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-12, 14-17, and 21 drawn to a polynucleotide of SEQ ID NO: X, vectors, host cells, polypeptides, methods of making polypeptides and methods of making host cells.

Group II, claim 13, drawn to an antibody.

Group III, claim 18, drawn to a method of diagnosis comprising the detection of a mutation in a polynucleotide.

Group IV, claim 19, drawn to a method of diagnosis comprising measuring the amount of a polypeptide.

Group V, claim 20, drawn to a method of identifying a binding partner of a polypeptide.

Group VI, claim 22, drawn to a method for identifying a biological activity.

Group VII, claim 23, drawn to a product.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

In Group I, SEQ ID NO: X corresponds to any of 31 different amino acid sequences listed in the Table beginning on page 91.

The inventions listed as Groups I-VII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Pursuant to 37 C.F.R. 1.475(d), this authority considers that the main invention in the instant application comprises the first recited product, a polynucleotide of SEQ ID NO: 11, and the first recited method of using that product, namely in the process of producing the encoded polypeptide. Further, pursuant to 37 C.F.R. 1.475(b)-(d), the ISA/US considers that the materially and functionally dissimilar products of Groups II and VII and the additional methods of Groups III and VI do not correspond to the main invention. This authority therefore considers that the several inventions do not share a special technical feature within the meaning of PCT Rule 13.2 and thus do not relate to a single general inventive concept within the meaning of PCT Rule 13.1.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: The different polypeptides of Group I are distinct molecules with different structures and features.